REPORTS

OF THE

COMMISSION

APPOINTED BY

THE ADMIRALTY, THE WAR OFFICE, AND
THE CIVIL GOVERNMENT OF MALTA,

FOR THE INVESTIGATION OF

MEDITERRANEAN FEVER,

UNDER THE SUPERVISION OF AN

ADVISORY COMMITTEE

OF

THE ROYAL SOCIETY.

PART I.

LONDON:
HARRISON AND SONS, ST. MARTIN'S LANE,
PRINTERS IN ORDINARY TO HIS MAJESTY.

MARCH, 1905.

CONTENTS.

INTRODUCTION.

The Mediterranean Fever Commission had its origin in a letter from Mr. Secretary Lyttelton, dated January 25, 1904, addressed to the Royal Society, in which he states that his attention has recently been called to the prevalence of Mediterranean fever in Malta among the Naval and Military forces, as well as the civil population.

It accordingly appeared to him to be desirable that the investigation of this fever should be taken in hand, and he addressed a despatch to the Governor of Malta proposing the appointment of a joint Commission representing the Army, the Navy, and the Civil Government.

He enclosed a copy of a despatch from the Governor in reply, entirely concurring in the proposed appointment of a joint Commission. The War Office and Admiralty also expressed their concurrence in the proposal.

Mr. Secretary Lyttelton then went on to say that the War Office, the Admiralty, and the Civil Government desired to secure for this Commission the assistance of the Royal Society, and asked whether the Society would be willing to appoint an Advisory Board of experts in this country for the purpose of supervising the investigations.

In reply to this letter the Royal Society wrote, in February, 1904, consenting to nominate a Committee to direct the investigations, on the understanding that the selection of the investigators should be placed in the hands of the Royal Society.

A Sub-Committee of the Tropical Diseases Committee was accordingly formed, consisting of Colonel Bruce, R.A.M.C., Chairman, Fleet Surgeon Bassett-Smith, R.N., Dr. Klein, Dr. C. J. Martin, and Dr. Sidney Martin.

As it was desirable to begin the investigations with as little delay as possible, the Sub-Committee at once appointed Major Horrocks, R.A.M.C., Staff-Surgeon Shaw, R.N., and Dr. Zammit, Board of Health, Malta, as members of the Commission, and Colonel Bruce was requested to proceed to Malta to assist them in commencing the work. Colonel Bruce arrived in Malta on June 13, where he met the Commission, and work was at once begun. He remained in Malta until July 14, when he left for England. Dr. Johnstone, whose services were lent by the Local Government Board, on the application of the Royal Society, joined the Commission on June 30.

The best thanks of the Commission are due to the Governor, General Sir C. M. Clarke, G.C.B., and to the Lieutenant-Governor, the Hon. E. M. Merewether, C.M.G., for their courtesy and invaluable aid.

The following reports have been received, up to the present date, from the members of the Commission, and also one from Staff-Surgeon Gilmour, R.N., Bighi Hospital, Malta, who kindly placed his spare time at the service of the Commission.

1.

ON THE DURATION OF LIFE OF THE *MICROCOCCUS MELITENSIS* OUTSIDE THE HUMAN BODY.

(Experiments made at Gibraltar.)

By Major W. H. Horrocks, R.A.M.C., Member of Mediterranean Fever Commission.

(Received July 14, 1904.)

The small size and slow growth of the *Micrococcus melitensis* render the study of its saprophytic existence by no means an easy matter. In the hope of devising a medium which would simplify the isolation of the Micrococcus from a mixture of microbes, a careful study of its cultural characteristics on all modern media of an exact reaction was first made. It was thought that the degree of fermentation or non-fermentation of the various sugars might assist in attaining the desired differentiation. The results of the tests are shown in the following table :—

Cultural Characteristics.

Glucose peptone, 1 per cent.	Growth. Neither acid nor gas produced.
Lactose peptone, ,, ...	,, ,, ,,
Saccharose peptone, 1 per cent.	,, ,, ,,
Starch peptone, 1 per cent.	,, ,, ,,
Litmus milk.....	No clotting observed; at the end of 3 weeks the medium was found to have a distinctly alkaline reaction.
Peptone and salt solution.....	On the addition of a nitrite and pure sulphuric acid, the nitroso-indol reaction was never obtained.
Broth	Diffuse growth without any surface pellicle. After some days the broth cleared somewhat, and a deposit formed on the sides and at the bottom of the tube.
Agar slope	Greyish-white moist growth; discrete colonies, circular and transparent, resembling those of the Gram-staining streptococci found in fæces and urine. When the cultures are old, the growth often acquires a yellowish-brown colour.

Proskauer and Capaldi's media	No. I. No growth.
	No. II. Growth, but no change appeared in the reaction of the medium.
Neutral red	Unchanged after 48 hours at 37° C.; after 5 days' incubation a yellow colour appeared at the surface.
Potato	Moist transparent film appeared, and on scraping the surface a copious growth was obtained, the formation of chains being very marked. The reaction of the potato was made faintly alkaline by the addition of sodium carbonate, and on planting out on the surface a distinct yellowish coloured growth was obtained.
MacConkey's bile salt broth ...	Growth; reaction unchanged.
Nitrate broth	Growth, but no reduction of the nitrate occurred.
Gelatine stab and slope (22° C.)	Growth extremely slow; no liquefaction of the medium.
Agar stab (37° C)	Diffuse growth.
Anaërobiosis	Growth, but more feeble than under aërobic conditions.
Morphology	Very small cocci, appearing as diplococci and short chains; occasionally chains of twelve to fourteen cocci were observed.

The failure of the *M. melitensis* to ferment glucose, and its power of rendering milk alkaline are very important cultural reactions. The Gram-staining streptococci, isolated from sewage, urine, fæces, cases of erysipelas, and from septic throats, all ferment glucose; the amount of acid produced, however, is a variable quantity. In glucose agar media, tinted with litmus, the Gram-staining streptococci produce colonies varying in tint from a rose red to a bright red, but the colonies of the *M. melitensis* are always blue, and after a few days' incubation the colour deepens in tint.

The gelatine, broth, agar, and peptone media, were made with a reaction of + 10 (Eyre's scale), and as a rule distinct growth was not observed until the 2nd or 3rd day after planting out, incubation being at 37° C.

Several observers having stated that the *M. melitensis* grew best on media having an alkaline reaction; batches were prepared having reactions: − 15, − 10, neutral, + 10, + 15 (Eyre's scale). Approximately, the same amount of culture was planted out, and it was found that the quickest and most copious growth was obtained on the + 10 medium; on the − 10 and − 15 there was practically no growth.

Having determined the most favourable reaction, trials were made to see if a medium could not be obtained on which the *M. melitensis* would grow in 24 hours. Bearing in mind the favourable effect of nutrose on the growth of *B. typhosus*, a 1-per-cent. nutrose agar, + 10,

was prepared, and on this a marked growth of *M. melitensis* occurred in 16 hours. A similar vigorous growth was obtained in nutrose broth.

The study of the cultural reactions having shown that the *M. melitensis* did not ferment glucose, it appeared that the addition of this sugar to the nutrose medium, tinted with litmus, would be of great service when isolating the organism from a mixed culture. As previously stated, the Gram-staining streptococci, which occur in urine and fæces, ferment glucose, forming enough acid to change the blue medium to a rose tint, and as the colonies of these organisms have much the same transparent appearance as those of *M. melitensis* on nutrose agar, the use of the glucose litmus medium enables a separation to be readily made, and saves much time when investigating plate cultures.

Trials were then made with the *M. melitensis* added to non-sterile water and soil, and it was found that the organism could be readily isolated when it was present in considerable quantity; when, however, only a few cocci were present, there was a marked tendency for the water and soil organisms to grow over the plate, the nutrose evidently accelerating the growth of these organisms. Accordingly, attempts were made to restrain the growth of these organisms by the addition of sodium taurocholate, carbolic acid, malachite green, etc.

A medium containing 0·5 per cent. sodium taurocholate, 1 per cent. peptone and 0·5 per cent. salt was prepared, and the tubes inoculated with *M. melitensis*, urine, soil, and water respectively. The results are shown in the following table:—

		24 hours.	48 hours.	72 hours.	96 hours.
Tube 1.	*M. melitensis*	±	±	+	+
Tube 2.	One loop urine	±	±	+	+
Tube 3.	One loop of soil	±	±	+	+

Note.—±, feeble growth; +, good growth; −, no growth.

The growth which appeared in Tube 1, after 48 hours' incubation, was planted out on nutrose agar, and the *M. melitensis* recovered after 3 days' incubation at 37° C.

This experiment showed that, while the sodium taurocholate restrained the growth of the microbes in soil and urine, it had also a marked inhibiting effect on the growth of the *M. melitensis*.

The addition of nutrose to the taurocholate medium was then tried, with the following result:—

	24 hours.	48 hours.	72 hours.
Tube 1. *M. melitensis*	±	+	+
Tube 2. One loop of urine ...	+	+	+ +
Tube 3. One loop of soil	±	+	+

The growth in Tube 1, which appeared in 48 hours, was planted out on nutrose agar, and the *M. melitensis* recovered after 48 hours' incubation at 37° C.

The addition of the nutrose caused a more vigorous growth of the *M melitensis*, but unfortunately the growth of the bacteria in urine was enhanced more than that of the *M. melitensis*. The results with these media when grown at 37° C. being unsatisfactory, the temperature of incubation was raised to 42° C. in the hope that it might cause a more satisfactory separation. Hughes, in his book on Mediterranean fever, stated that the *M. melitensis* would not grow at 42° C., so a preliminary planting out on ordinary agar and nutrose agar was tried. The results were as follows :—

	24 hours.	48 hours.	72 hours.	96 hours.	5 days.
Ordinary agar (+10)	−	−	−	±	±
Nutrose agar (+10)	±	±	±	+	+

Temperature of incubation, 42° C.

On ordinary agar the growth was much delayed and feeble at the end of 5 days, but on nutrose agar a good growth was obtained after 72 hours.

Nutrose, sodium taurocholate peptone tubes were now inoculated with soil, urine, tap-water and *M. melitensis*. Incubation 42° C.

	24 hours.	48 hours.	72 hours.	96 hours.
Tube 1. One c.c. tap-water ..	−	±	±	+
Tube 2. One loop soil	−	±	+	+ +
Tube 3. One of urine	±	+	+	+ +
Tube 4. One of *M. melitensis*.	±	±	±	+

The results were again disappointing; the method would be of very little use in regard to urine investigation, but might render some assistance when working with inoculated water supplies.

Malachite green, krystal violet, etc., being credited with the power of restraining the growth of saprophytes, the former salt was selected for experiment.

The powder was dissolved in distilled water and the solution added to +10 broth, so as to make dilutions of 0·01 per 1,000, 0·02 per 1,000, 0·05 per 1,000, 0·1 per 1,000, and 0·2 per 1,000. The tubes were incubated at 37° C. for 24 hours, and remaining quite sterile were each inoculated with one loopful of an emulsion of *M. melitensis*. After 24 hours' incubation at 37° C., it was found that there was a good growth of *M. melitensis* in all the tubes except the 0·2 per 1,000. Similar dilutions were then inoculated with urine and soil—the tube containing 0·1 per 1,000 was found to have a marked restraining influence on the growth of the bacteria for a period of 24 hours; but after 48 hours' incubation there was a rapid growth of the bacteria in urine.

Nutrose was then added to the malachite green solution, so that the medium now contained 1 per cent. of nutrose and 0·1 per 1,000 of malachite green.

The tubes were inoculated with an emulsion and incubated at 37° C. After 24 hours it was found that there was a vigorous growth of the *M. melitensis*, but unfortunately, as in the case of the sodium taurocholate, the bacteria in the urine and soil also showed a marked growth. Consequently, it was decided to omit the nutrose from the malachite green broth during the preliminary investigations. A non-sterilised garden soil was inoculated with *M. melitensis* and then planted out in malachite green broth; after 24 hours' incubation at 37° C. a feeble growth occurred, which was stroked over the surface of a series of Petri dishes containing nutrose agar. The plates were incubated at 37° C.; after 24 hours there was practically no growth, but after 48 hours there was a marked growth, and the transparent colonies of the *M. melitensis* were easily detected scattered amongst the larger and opaque colonies produced by the soil organisms. This result was satisfactory, and the procedure appears likely to give useful results.

Carbolic acid was next tried; it was found that the *M. melitensis* grew well in 24 hours in 0·05 per cent. carbolic broth, but this small amount of acid has a very slight restraining influence on the growth of the bacteria in urine and soil, and consequently the *M. melitensis* was always crowded out by the saprophytic bacteria. The amount of carbolic acid was increased to 0·1 per cent., but in this the *M. melitensis* did not appear for 4 days, whereas the saprophytic organism grew vigorously in 48 hours. Accordingly, carbolised media were abandoned during the research.

Exposure to a temperature of 42° C., and the presence of malachite green, carbolic acid and sodium taurocholate, having failed to restrain the growth of bacteria present in urine obtained from Malta fever

patients after careful sterilisation of the external parts, growth under anaërobic conditions was tried but with equally unsatisfactory results. It now appeared evident that in the study of urine all restraining influences must be abandoned and efforts made to obtain as free a growth of the microbes as possible, trusting to subsequent dilution to obtain isolated colonies for purposes of study. Experimentally, this procedure succeeded well enough when the Micrococcus was added in considerable quantity to urine, but when the amount inoculated was small, isolation of the Micrococcus could not be effected. Trials were then made as to the effect of adding a strong specific serum to these latter growths; it was thought that the serum might cause the aggregation of the Micrococci into clumps, and if these were planted out on agar plates a better chance of success might be obtained. The results were encouraging, and in future examinations of the urine of Malta fever cases, it is intended to follow this procedure, as well as the usual dilution method on agar plates.

Experiment I.

An investigation was now undertaken to ascertain whether the *M. melitensis* could live in urine, and especially in a urine which had become alkaline from the decomposition of urea.

A freshly passed urine from a healthy man was inoculated with an emulsion of *M. melitensis* made in distilled water from a recent agar slope. The urine when passed appeared practically sterile. The inoculated urine was placed in a laboratory cupboard and examined daily by plating on nutrose agar. The Micrococcus was easily recovered up to and on the 6th day, but could not be detected at a later period. The urine on the 6th day was markedly alkaline from the presence of ammonia, and on titrating it with N/10 acid, the ammonia was found to equal 0·0064 gramme NH_3 per c.c.

This result is of some practical importance as it shows that the *M. melitensis* might be recovered from a urine which had been kept for 6 days and become alkaline in reaction.

The viability of the *M. melitensis* in the presence of ammonia and the comparative absence of saprophytic microbes from the urine in the experiment just related, suggested that, perhaps, this alkali might have a restraining influence on the growth of the bacteria usually found in the urine of Mediterranean fever cases, and so assist in the separation of the specific microbe. Accordingly, broth (+ 10) was treated with pure NH_3 until the amount when titrated with N 10 acid equalled 0·64 gramme per litre. The tubes were incubated and remaining sterile, were then inoculated with *M. melitensis* and with urine from a case of Mediterranean fever. After 24 hours' incubation there was a marked growth of bacteria in the tubes inoculated with urine, but the *M. melitensis* did not show any marked growth until the 4th day. The

result was not unexpected as the work previously done on the reaction of media had shown that the *M. melitensis* did not grow well in alkaline media.

Experiment II.

This experiment was designed in order to ascertain the duration of life of *M. melitensis* when maintained in an absolutely dry state and without a trace of nutrient medium.

A series of sterile cover glasses were placed in a Petri dish and then inoculated with an equal quantity of an emulsion of *M. melitensis*, the cocci from a 48 hours' agar slope being suspended in water. The emulsion was exposed to the air until all traces of moisture had disappeared from the cover glasses. The Petri dish was then placed in a laboratory cupboard, the temperature of which averaged 18° C. Every 24 hours a cover slip was removed and planted out in broth. The resulting growth was plated on agar, and the colonies fished and examined in the following manner:—A likely colony was made into an emulsion with a loopful of broth and then examined under $\frac{1}{12}$th objective; if cocci were found freely disseminated through the field and showing no signs of clumping, a loopful of serum from an inoculated rabbit was added. When clumping occurred the needle, which had been used to make the emulsion and *not* sterilised, was rubbed over an agar slope. The resulting growth was planted out in glucose peptone, lactose peptone, cane sugar peptone, litmus milk, peptone and salt solution, nitrate broth, and stabbed into gelatine. The growths which resulted corresponded exactly to those obtained when the original *M. melitensis* was planted out in these media.

Result.—A Micrococcus, which corresponded in every particular to the *M. melitensis*, was isolated up to and on the 16th day.

Experiment III.

The object of this experiment was to ascertain the duration of the life of *M. melitensis* in dry soil.

Some soil from a recently manured plot of ground in Gibraltar was powdered, dried, and sterilised, and then inoculated with an aqueous emulsion of *M. melitensis* prepared from an agar slope. The soil was allowed to dry naturally and kept in the laboratory cupboard mentioned in the previous experiment. For a few days traces of moisture were present, but after the 10th day the soil was quite light and formed a black powder which could easily be blown about. The soil was tested weekly for the presence of *M. melitensis*, a portion of the soil being planted out in broth and the resulting growth treated in the manner detailed under Experiment II. Up to and on the 69th day a Micrococcus was recovered, corresponding in every way to the *M. melitensis* originally planted out. During this experiment

careful watch was kept for any change in the morphology of the inoculated microbe. It was thought that the bacillary forms described by Durham might appear, and cause some difficulty in diagnosing the culture. The bacillary forms were never seen, and the Micrococcus obtained on an agar slope on the 69th day presented the usual morphology. The cultural characteristics and reaction to the specific serum were also unchanged.

Result.—The *M. melitensis* retained its vitality in dry soil for 69 days.

Experiment IV.

In this experiment a fine sterile sand, practically free from organic matter, was inoculated, and treated in exactly the same manner as the manured soil in Experiment III. The *M. melitensis* was recovered up to and on the 20th day, but not later. The morphology, cultural and serum reactions, were again quite unchanged.

Result.—The *M. melitensis* retained its vitality in dry sand for 20 days.

Experiment V.

The object of this experiment was to discover the duration of life in a foul soil saturated with water. The manured sterile soil employed in Experiment III was inoculated in the same manner as before, but instead of being allowed to dry it was kept saturated with sterile tap-water. The *M. melitensis* was recovered up to and on the 7th day, but could not be detected at a later date, although many trials were made. The result of this experiment seemed to show that immersion in water was inimical to the persistence of the *M. melitensis* in a saprophytic condition.

Result.—The *M. melitensis* retained its vitality in a foul, saturated soil for 7 days.

Experiment VI.

The idea of this experiment was to ascertain the duration of life of the *M. melitensis* when dried on fabrics. Accordingly, pieces of thick regulation blanket, khaki serge, and khaki cotton were inoculated with an emulsion of the microbe made by suspending a recent agar growth in sterile water. The greatest care was taken not to remove any of the nutrient medium. After inoculation the infected fabrics were placed in a Petri dish and allowed to dry naturally; they were then placed in the laboratory cupboard during the whole experiment. Portions of the fabrics were planted out in broth every 3 or 4 days, and the resulting growth plated on nutrose agar in the usual manner. The *M. melitensis* was recovered from the khaki cotton up to and on the 80th day, from the khaki serge on the 80th day, and from the blanket on the 80th day. The morphology, cultural and serum reactions, were again quite unchanged.

Experiment VII.

The rapid disappearance of the *M. melitensis* from the soil saturated with water suggested that an attempt should be made to determine the duration of life of the *M. melitensis* in sterile water. The whole of a recent growth from an agar slope was diffused in 50 c.c. of sterile tap-water, representing an exceedingly gross pollution. The flask was kept in the laboratory cupboard, and every day 1 c.c. was plated on nutrose agar. The Micrococcus was readily isolated for 6 days, but on the 7th and 13th days it could not be detected.

Experiment VIII.

This experiment was a repetition of Experiment VII, but instead of planting out small quantities from day to day, the flask was left undisturbed for 3 weeks. Broth was then added so as to enrich the whole bulk of the water, and the flask incubated at 37° C. for 3 days. The growth which resulted was found to contain numerous small cocci decolorised by Gram's method. A portion of the growth was then added to an equal quantity of a strong rabbit serum diluted 1—10, and the whole thoroughly mixed was drawn up into a capillary pipette. Distinct agglutination having occurred, the pipette was then opened and the agglutinated mass stroked over a series of agar plates; unfortunately a pure culture of the *M. melitensis* was not obtained. The result of this experiment is not conclusive, but it suggests that the duration of life of the *M. melitensis* in water may be longer than 1 week.

Conclusions.

(1) The *M. melitensis* is able to live for 6 days in a urine which has become alkaline from the presence of ammonia.

(2) The *M. melitensis* survives for 16 days when spread in a thin layer on a glass cover slip.

(3) The *M. melitensis* survives for 69 days when planted in a dry sterilised manured soil.

(4) In dry sterilised sand the duration of life of the *M. melitensis* appears to be only 20 days.

(5) In a sterilised manured soil saturated with water the *M. melitensis* appears to survive for only 7 days.

(6) The *M. melitensis* is able to live for 80 days on dry fabrics, such as blanket, khaki serge, and khaki cotton.

(7) The *M. melitensis* appears to live for a comparatively short time in sterilised tap-water. It was only recovered in pure culture 6 days after being planted out, though from the result of Experiment VIII it appears possible that the duration of life may extend to 3 weeks.

2.

FURTHER STUDIES ON THE SAPROPHYTIC EXISTENCE OF *MICROCOCCUS MELITENSIS*.

By Major W. H. Horrocks, R.A.M.C., Member Mediterranean Fever Commission.

(Received September 17, 1904.)

1. Duration of Life of the *M. melitensis* in Sterilised Tap-Water.

Experiment I.

In the Gibraltar report it was stated that the duration of life of the *M. melitensis* in sterilised tap-water was probably longer than the recorded experiments indicated. Accordingly, the experiment of adding an emulsion of *M. melitensis*, made by carefully mixing the growth from an agar slope in sterile water to a known volume of water, was repeated. In this case 1 c.c. of an emulsion made from a strain of *M. melitensis* isolated from urine was added to 10 c.c. of sterilised tap-water. Chemical analysis showed that the tap-water was very pure, and contained practically no organic material. The emulsion was added to the tap-water on August 1, 1904, and at various times 0·5 c.c. was removed, and added to 10 c.c. of broth, the contents of the tube being thoroughly mixed and then incubated at 37° C. As soon as the broth tube showed any signs of growth a large loopful was stroked in a zig-zag manner over an agar slope, which was then incubated at 37° C. On August 15, 1904, a pure culture of *M. melitensis* was isolated, the growth responded to all the usual cultural tests, and agglutinated at once with the serum of Monkey 45, diluted 1—1000. On August 21, 1904, the same procedure was followed, and the *M. melitensis* again isolated. On August 27, 1904, a pure culture of *M. melitensis* was obtained, and appeared quite unchanged. On September 6, 1904, the specific microbe was again isolated.

Result.—The *M. melitensis*, derived from urine, appears to survive for 37 days in sterilised tap-water.

2. Duration of Life of the *M. melitensis* when Planted out in Soil.

In the Gibraltar experiments already recorded a manured garden soil and a dry sand were employed. Valletta and Sliema are mainly built on the Globigerina limestone, and the white dust which abounds on the roads is chiefly due to the attrition of this stone; occasionally the soil has a red colour, due to the presence of oxide of iron resulting from the oxidation of FeS_2 (iron pyrites).

Experiment II.

A grey coloured soil was obtained from Sliema, and ground into a fine powder. According to Sir John Murray's analysis, this soil has the following composition:—

Carbonate of lime, iron, and alumina ($CaCO_3$,Fe_2O_3,Al_2O_3)	78·39
Phosphate of lime ($Ca_3 2PO_4$)	2·70
Magnesium carbonate ($MgCO_3$)	0·44
Calcium sulphate ($CaSO_4$)	0·33
Insoluble in dilute HCl (1—10)	17·87
	99·73

The soil was carefully dried and sterilised, and a portion planted out in broth and incubated at 37° C. After 4 days' incubation there were no signs of growth, showing that sterilisation had been effected. On July 15, 1904, the soil was inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope in distilled water, and allowed to dry naturally. On July 23, 1904, a portion of the soil, still showing faint traces of moisture, was planted out in broth and incubated at 37° C. On July 26, 1904, a growth occurred in the broth culture, which was planted out on an agar slope; two days later a typical growth, which responded to all the characteristic tests, appeared. On July 30, 1904, the soil was noted to be practically dry. On August 11, 1904, a portion of the soil was removed and treated in the same manner as on July 23, 1904; a typical growth of the *M. melitensis* was again obtained. On August 19, 1904, the same procedure was followed, and a pure culture of the specific microbe was isolated. On August 27, 1904, the *M. melitensis* was again isolated.

Result.—The *M. melitensis* survived for 43 days in a soil, which was allowed to dry naturally, and which was free from appreciable traces of moisture for 27 days.

Experiment III.

In this experiment a reddish coloured soil, also obtained from Sliema, was employed. Sir John Murray's analysis of this soil gave the following results:—

Carbonate of lime ($CaCO_3$)	80·24
Phosphate of lime ($Ca_3 2PO_4$)	3·57
Magnesium carbonate ($MgCO_3$)	1·63
Calcium sulphate ($CaSO_4$)	0·06
Iron and alumina (Fe_2O_3 and Al_2O_3)	1·13
Insoluble in dilute HCl (1 in 10)	12·88
	99·51

The soil was sterilised, and its sterility tested as in Experiment I. On June 25, 1904, it was inoculated with an emulsion of *M. melitensis*, made in sterile water from an agar slope grown for 48 hours at 37° C. The soil, having been dried in the incubator at 37° C., was placed in the laboratory cupboard. On July 4, 1904, a portion of the soil was planted out in broth, and the growth which resulted on July 7, 1904, was planted out on an agar slope. A typical culture, giving all the reactions of the *M. melitensis*, was obtained.

On July 11, 1904, the soil was again tested, and a pure culture of *M. melitensis* was isolated.

On July 15, 1904, an examination was made, but the growth in broth did not take place for 9 days, showing that the organism was much enfeebled. On planting out the growth on agar only a few colonies of the *M. melitensis* were obtained. On July 24, 1904, and on July 30, 1904, examinations were made, but the results were negative, the *M. melitensis* having apparently died out.

Result.—The *M. melitensis* lived for 21 days in red Sliema soil, thoroughly dried immediately after inoculation.

Experiments IV and V.

These experiments were designed in order to ascertain whether the presence of traces of moisture, as distinguished from flooding of the soil, had any influence on the survival of the *M. melitensis*.

In Experiment IV white Globigerina limestone dust was inoculated with *M. melitensis* on July 8, 1904; the tube was then placed in the laboratory cupboard. About once a week a little sterile tap-water was added by means of a pipette, so as to preserve a faint appearance of moisture on the surface of the soil. At various intervals portions of the soil were removed and planted out in broth, the tube being then incubated at 37° C. The resulting growth was planted on agar and tested as already described under Experiment I.

The *Micrococcus melitensis* was isolated on July 15, 1904.
" " " July 24, 1904.
" " " July 30, 1904.
" " " August 11, 1904.
" " " August 19, 1904.

The Micrococcus melitensis was isolated on August 27, 1904.
" " " September 7, 1904.
" " " September 19, 1904.

Result.—The *M. melitensis* survived for 72 days in a damp soil.

In Experiment V the red soil, described under Experiment II, was employed. The soil was inoculated on July 8, 1904, and the testings carried out at the same time as in Experiment III. The *M. melitensis* was isolated after 72 days' immersion in this soil.

3. Survival of the *M. melitensis* after Exposure to the Sun.

Experiment VI.—Exposure on Thin Strips of Glass.

A 36-hours' growth of *M. melitensis* on nutrose agar was made into an emulsion with sterile tap-water. A series of thin glass cover slips were sterilised and the surface of each inoculated with the emulsion by means of a sterile pipette. The cover slips were then exposed to the sun as follows :—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. (56°1 C.).

After each exposure one of the cover slips was added to sterile broth and incubated at 37° C. The broth tubes all remained sterile, though the incubation was maintained for 14 days.

From control slips, not exposed to the sun, the *M. melitensis* was easily recovered.

Experiment VII.—Exposure in a Very Thin Layer of Soil.

Samples of white and red soils, already mentioned under the soil experiments, were spread in layers, $\frac{1}{8}$ inch deep, on the bottom of glass dishes, and then inoculated with an emulsion of *M. melitensis*, made from an agar slope as mentioned above. The dishes were exposed to the sun as follows :—

On June 20, 1904, from 12.15 P.M. to 1 P.M. Maximum temperature in the sun, 128° F. (53°·3 C.).

On June 21, 1904, from 8.50 A.M. to 11.50 A.M. Maximum temperature in the sun, 135° F. (57°·2 C.).

On June 22, 1904, from 8.45 A.M. to 11.45 A.M. Maximum temperature in the sun, 126° F. (52°·2 C.).

On July 1, 1904, from 10.30 A.M. to 12.30 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each experiment particles from the dried baked surface were planted out in broth, and any resulting growth was then planted out on agar and the growth tested for agglutination, etc. The *M. melitensis* was recovered after the exposure on June 21, 1904, representing $3\frac{3}{4}$ hours' exposure to direct sunlight, but not later.

The *M. melitensis* was readily obtained from a control soil after 21 days in the laboratory cupboard.

Experiment VIII.—Exposure on Khaki Drill.

A piece of khaki drill was inoculated with the same emulsion used in the previous experiments. The drill was then exposed to the sun as follows:—

On June 17, 1904, from 9.30 A.M. to 11 A.M. Maximum temperature in the sun, 130° F. (54°·4 C.).

On June 17, 1904, from 3.10 P.M. to 4.10 P.M. Maximum temperature in the sun 130° F. (54°·4 C.).

On June 19, 1904, from 10.15 A.M. to 12.15 P.M. Maximum temperature in the sun, 133° F. (56°·1 C.).

After each exposure a portion of the infected drill was cut off and planted out in broth, and the resulting growth planted out on agar and tested in the usual manner.

The *M. melitensis* was recovered after an exposure of not more than $2\frac{1}{2}$ hours to the sun.

Experiment IX.—Exposure on Soil $\frac{1}{2}$-inch Deep.

The idea of this experiment was to ascertain whether the deeper layers of the soil, which were quite dry and capable of being blown about by strong winds, would still retain infection after exposure to the sun.

The white Globigerina limestone soil, previously described, was sterilised and carefully poured into a sterile Petri dish so as to form a uniform layer $\frac{1}{2}$ inch deep. The soil was then inoculated with an emulsion of *M. melitensis*, made by suspending the growth on an agar slope, inoculated from a urine culture and incubated for 48 hours at 37° C. The soil was exposed to the sun as follows:—

August 19, 1904, 3.30 P.M. to 4.30 P.M. Maximum temperature in the sun, 147° F. (63°·8 C.).

August 20, 1904, 9 A.M. to 11.45 A.M. Maximum temperature in the sun, 153° F. (67°·2 C.). After the total exposure of $3\frac{3}{4}$ hours, a portion from the surface was planted out in broth, so as to compare this experiment with the one previously reported.

August 21, 1904, exposed from 9.30 A.M. to 11.30 A.M. Maximum temperature in sun, 154° F. (67°·7 C.). After a total exposure of

5¾ hours, portions of soil taken from the surface and from the depth were planted out in broth tubes.

August 22, 1904, exposed from 9 A.M. to 11.15 A.M. Maximum temperature in sun 148° F. (64 ·4 C.). Portions of soil from the surface and depth again planted out in broth.

August 23, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 148° F. (64°·4 C.). Planted out portions of soil from the surface and depth in broth tubes.

August 25, 1904, exposed from 10.15 A.M. to 11.15 A.M. Maximum temperature in the sun, 146° F. (63 ·3 C.). Total exposure since the 19th equals 10 hours. Planted out portions of soil from the surface and depth in broth tubes.

September 6, 1904. All the broth tubes which had been incubated at 37 C., since the date of inoculation, were planted out on agar slopes.

September 12, 1904. All the agar tubes inoculated with the broth containing the surface soil, have remained quite sterile.

September 12, 1904. The agar tubes inoculated with the broth containing the portions of soil taken from the depth after 5¾ and 8 hours' exposure, show a growth of *B. mesentericus*. There is no sign of the *M. melitensis*.

The agar tubes inoculated with the broth tubes containing the soil from the depth after 9 and 10 hours' exposure are quite sterile.

Result.—The heat derived from exposure to the sun, the maximum temperature varying between 146° F. and 153° F., apparently destroys the *M. melitensis* at a depth of ½ inch from the surface.

Experiment X.—Duration of Life of the M. melitensis *when Planted out in Sea-Water.*

Sea-water was obtained from the harbour and sterilised. A portion was then planted out on agar and in broth; both the tubes remained sterile after incubation at 37° C.

On July 25, 1904, a tube containing 10 c.c. of sterile sea-water was inoculated with the growth obtained from an agar slope, incubated for 13 days at 37° C. The inoculated tube was placed in the laboratory cupboard. On July 29, 1904, 0·5 c.c. was removed from the tube and planted out in broth; on September 2, 1904, there was a distinct growth in the broth; the growth was planted out on an agar slope, and a typical growth of *M. melitensis* was obtained, which responded to the classical tests.

On July 31, 1904, 0·5 c.c. was planted out in broth, and the same procedure followed as on July 29, 1904; a typical growth of *M. melitensis* was obtained.

On August 5, 1904, 0·5 c.c. was planted out in broth; a growth of *M melitensis* resulted.

On August 8, 1904, 0·5 c.c. was planted out as before, and a pure culture of *M. melitensis* was obtained.

On August 12, 1904, 0·5 c.c. was planted out in broth; the resulting growth when planted on an agar slope gave rise to a growth, which agglutinated very slowly with the serum from Monkey 45. A portion of the growth was planted out in glucose and litmus milk; the glucose was not fermented, and the litmus milk became alkaline, without showing the slightest trace of coagulation or digestion. The growth also had a typical morphology, and did not stain by Gram's method.

On August 15, 1904, 0·5 c.c. was planted out in broth, and a culture again obtained, which was typical of *M. melitensis*, except that the agglutination occurred slowly.

On August 19, 1904, 0·5 c.c. was planted out, and the same result obtained as on August 12 and 15, 1904. The growth was tested with the specific serum which, diluted 1—1000, caused instantaneous agglutination of the laboratory standard culture of *M. melitensis*. With the growth from sea-water, this serum, diluted 1—1000, caused clumping in $\frac{1}{2}$ hour.

On August 22, 1904, 0·5 c.c. was planted out in broth, and incubated at 37° C. No sign of growth appeared after 15 days' incubation.

On August 26, 1904, 0·5 c.c. was again planted out, but no growth appeared.

Result.—The *M. melitensis* appears to survive for 25 days in sterilised sea-water.

Conclusions.—1. The *M. melitensis* retains its vitality in sterilised tap-water for 37 days.

2. In a Maltese soil, allowed to dry naturally, the *M. melitensis* survives for 43 days; and in one thoroughly dried immediately after inoculation, it survives for 21 days.

3. The *M. melitensis* survives for 72 days in a damp soil.

4. Exposure to the sun for a few hours kills the *M. melitensis*.

5. The *M. melitensis* survives for 25 days in sterilised sea-water.

3.

ON THE RECOVERY OF THE *MICROCOCCUS MELITENSIS* FROM THE URINE, FÆCES, AND SWEAT OF PATIENTS SUFFERING FROM MEDITERRANEAN FEVER.

By Major W. H. Horrocks, R.A.M.C., Member Mediterranean Fever Commission.

(Received September 17, 1904.)

Note.—The work on the examination of urine, fæces and mosquitoes has been done in conjunction with Captain Kennedy, R.A.M.C.

1. *Examination of Urine.*

In my report on previous work performed at Gibraltar, it was pointed out that the ordinary restraining agents, such as carbolic acid, sodium taurocholate, malachite green, etc., could not be depended upon to inhibit the growth of the micro-organisms usually found associated with the *M. melitensis* in the urine of Mediterranean fever cases. Accordingly, in the earlier work at Malta, attempts were made to isolate the Micrococcus by first enriching a known bulk of urine with broth, usually in the proportions of 1—1 and 1—3, and then, after varying periods of incubation at 37° C., plating the growths, which resulted, on nutrose agar. It was hoped that, under these conditions, the specific microbe would so multiply as to enable colonies to be detected by the plate method. A very short experience showed that the enrichment method was not satisfactory; the extraneous organisms multiplied more vigorously than the *M. melitensis*, and the latter was completely crowded out. It was then decided to make use of the glucose-litmus-nutrose-agar plates, already mentioned in the Gibraltar report, and to add small quantities of urine, 0·25—0·33 c.c., to these plates, allowing the urine to flow over and form a thin layer on the surface of the solidified agar. This procedure enabled the actual number of colonies of the Micrococcus passed in the urine to be ascertained. Before collecting the urine for investigation, the genitalia were washed with carbolic acid lotion; the patient then passed urine, but the first portion, which acted as a flush to the urethra, was discarded. On the glucose-litmus-nutrose-agar plates, the colonies of the *M. melitensis* appeared as almost transparent deep blue drops; likely colonies were next fished, and made into an

emulsion with normal salt solution on a cover-glass. It may be noted that the *M. melitensis* readily emulsifies, and the culture appears to flow off the point of the needle into the surrounding fluid; this characteristic was found of great assistance in detecting the specific microbe. A streptococcus is found in urine which produces, on the special plates, colonies very closely resembling those of the *M. melitensis;* when fished, however, they do not readily emulsify, and, on examination, under one-twelfth, are found to consist of a medium-sized coccus, staining with Gram. When it was found that the colony readily emulsified, the hanging drop was carefully examined under the oil immersion, in order to ascertain the nature of the organism, and to make sure that no false clumps were present. If the microbe presented the characteristics of the *M. melitensis*, and the emulsion was satisfactory, the cover-glass was removed, and a little specific animal serum added. In the earlier work I employed a rabbit serum prepared at Gibraltar, but, in the later work, serum from Monkey 45 was used. When the microbe under examination manifested instantaneous clumping under the influence of the serum, a portion of the colony was planted out on an agar slope, and incubated at 37° C. The resulting growth was then treated as follows:—

(1) Tested with the serum of Monkey 45. This serum, when diluted 1—1000, was found to cause instantaneous clumping, visible to the naked eye, of the laboratory stock culture of *M. melitensis*.

(2) Planted in glucose-litmus-peptone, or on a glucose-litmus-agar slope, and incubated for 7 days at 37° C.

(3) Planted in litmus milk and incubated for a month at 37° C.

(4) Examined as to retention of stain by Gram's method.

A micro-organism, which agglutinates with a specific animal serum in a high dilution, does not ferment glucose, renders milk alkaline without coagulation, may justly be regarded as the *M. melitensis*.

All the strains of *M. melitensis*, which have been isolated from the urine of Mediterranean fever cases, have responded to these tests.

Employing the above technique the first successful isolation was obtained from the urine of Sergeant Pudney, 2nd Essex Regiment. A plate made with 0·33 c.c. of urine was found to contain thirty-three colonies, after 5 days' incubation at 37° C.; colonies were first observed on the 4th day, but the maximum number did not appear until the 5th day of incubation.

The *M. melitensis* has now been isolated thirty-nine times, and from the urine of thirteen different patients. Colonies were never observed before the 3rd day of incubation, and at this period they were usually very minute and easily missed; on the 4th day of incubation, however, they were readily detected on the glucose-litmus-nutrose-agar plates. The actual numbers of *M. melitensis* isolated from urine are shown in the attached table (A).

Table A.—Showing the Number of Colonies of *M. melitensis* found in each Sample of Urine.

Name.	Date.	Quantity of urine in c.c.	No. of colonies in each plate.	No. of colonies per c.c.	Average No. per c.c.
Howe	6.7.04	Isolated from broth culture made with urine obtained at the *post-mortem* examination.			
Pudney	18.7.04	0·5	1	2	
		0·33	33	99	50
,,	25.7.04	0·25	3		
		0·25	3	12	26
		0·25	3		
		0·25	3		
		0·25 (mucus)	21	84	
,,	27.7.04	0·33	2	6	6
Martin	2.8.04	0·33	95	285	285
Markham	6.8.04	0·25	5	20	20
Breuster	6.8.04	0·25	2	8	8
Belfield	6.8.04	0·33	1	3	3
Pudney	7.8.04	0·125	1	8	8
,,	8.8.04	0·5	4	8	8
Fisher	8.8.04	0·33	3	9	15
		0·33	7	21	
Lawson	12.8.04	0·25	1	4	4
Breuster	13.8.04	0·25	2	8	8
Lawson	14.8.04	0·33	4	12	12
,,	14.8.04	0·33	5	15	15
,,	15.8.04	0·25	6	24	24
Lawrence	16.8.04	0·25	1	4	4
		0·25	1	4	
Lawson	17.8.04	0·25	45	180	180
Fisher	19.8.04	0·25	1	4	4
Lawson	20.8.04	0·33	12	36	36
Griffin	20.8.04	0·25	10	40	40
Lawson	21.8.04	0·33	15	45	45
Griffin	23.8.04	0·25	1	4	4
Lawson	23.8.04	0·33	105	315	315
Pudney	23.8.04	0·33	2	6	6
Breuster	24.8.04	0·25	1	4	4
Griffin	24.8.04	0·25	1	4	4
Lawson	26·8.94	0·33	1	3	3
Markham	26.8.04	0·33	1	3	3
Lawson	29.8.04	0·33	3	9	9
Barry	29.8.04	0·33	1	3	3
Christie	29.8.04	0·33	4	12	12
Lawson	31.8.04	0·33	1	3	3
Lawrence	1.9.04	0·33	3	9	9
Markham	1.9.04	0·33	1	3	3
Griffin	9.9.04	0·33	5	15	15
Lawrence	10.9.04	0·33	70	210	210
Kinsella	16.9.04	0·33	1	3	15
		0·33	9	27	
Markham	28.9.04	0·5	298	596	596

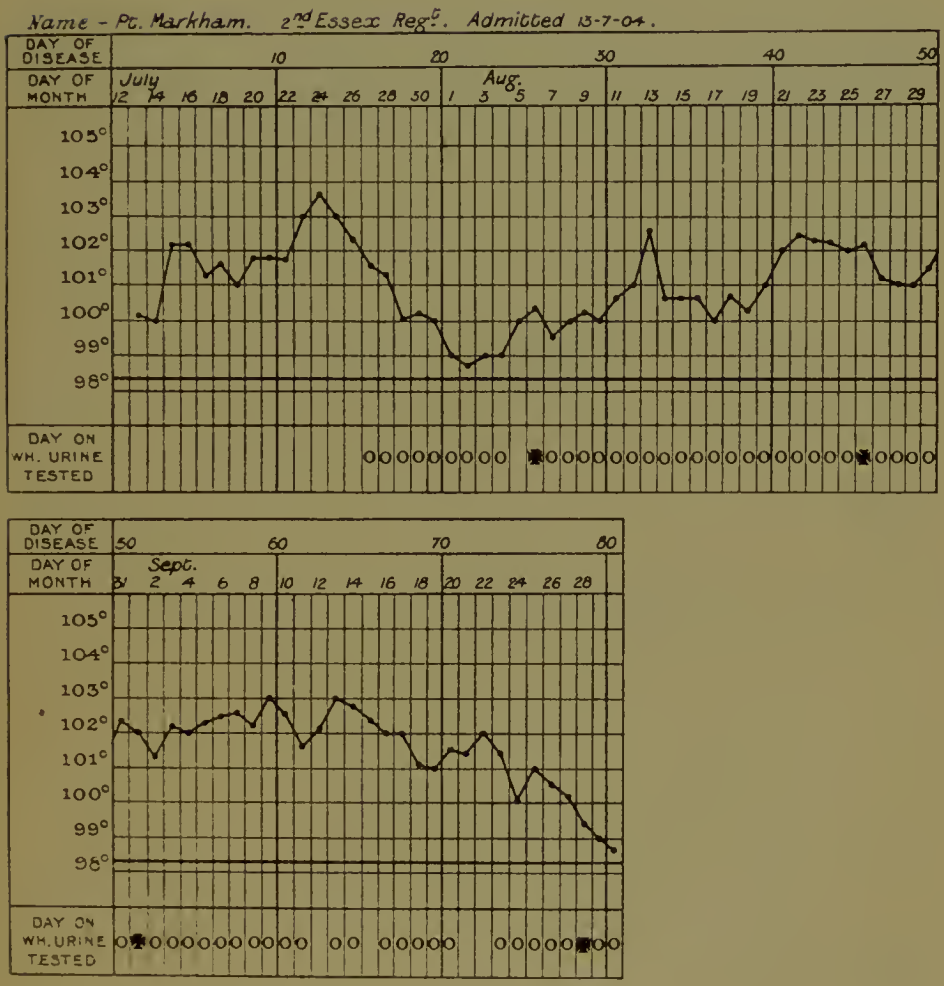
Up to the present time the Micrococcus has not been isolated from urine earlier than the 15th day or later than the 82nd day of disease.

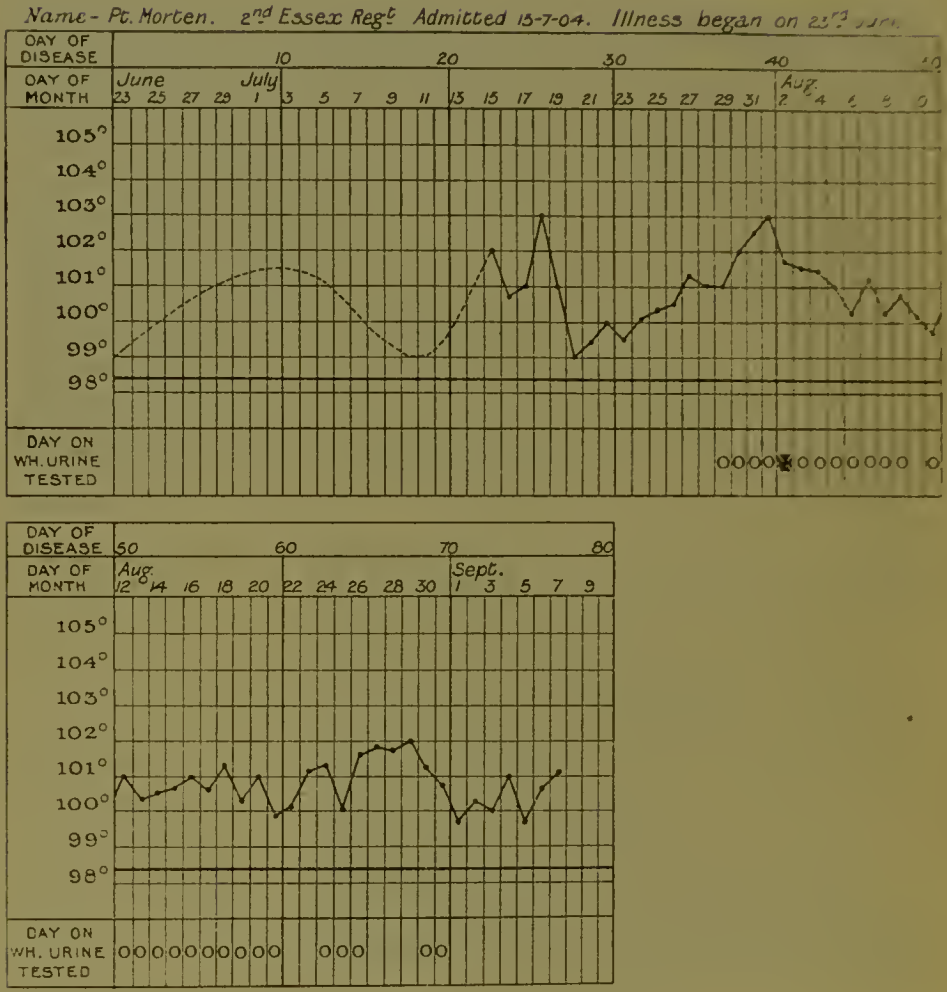
It is present in the urine of patients who are sufficiently convalescent to be allowed up, but still have an evening rise of temperature.

In order to save repetition and to enable the work done to be grasped at a glance, the attached charts have been prepared by Captain Kennedy, Royal Army Medical Corps, who has given me most valuable assistance throughout the work. Each square represents a day of disease, and in every case the chart commences with the day which, after careful questioning of the patient, was considered to be probably the 1st day of disease; so that on looking through the charts the different columns represent the same day of disease for each patient. The course of the fever is represented by the evening temperature, and the 0 sign indicates an examination made without any result; the Maltese cross sign represents a successful isolation of the *M. melitensis*. It will be noticed that there are many failures as compared with successes. In the earlier work the constant want of success was undoubtedly due to the faulty method of procedure; but in the later work it is to be attributed partly to the fact that the *M. melitensis* is not voided in the urine every day, but appears in gushes at uncertain periods, and partly to the presence in the urine of acid-producing organisms, which outgrow and interfere with the development of the *M. melitensis*.

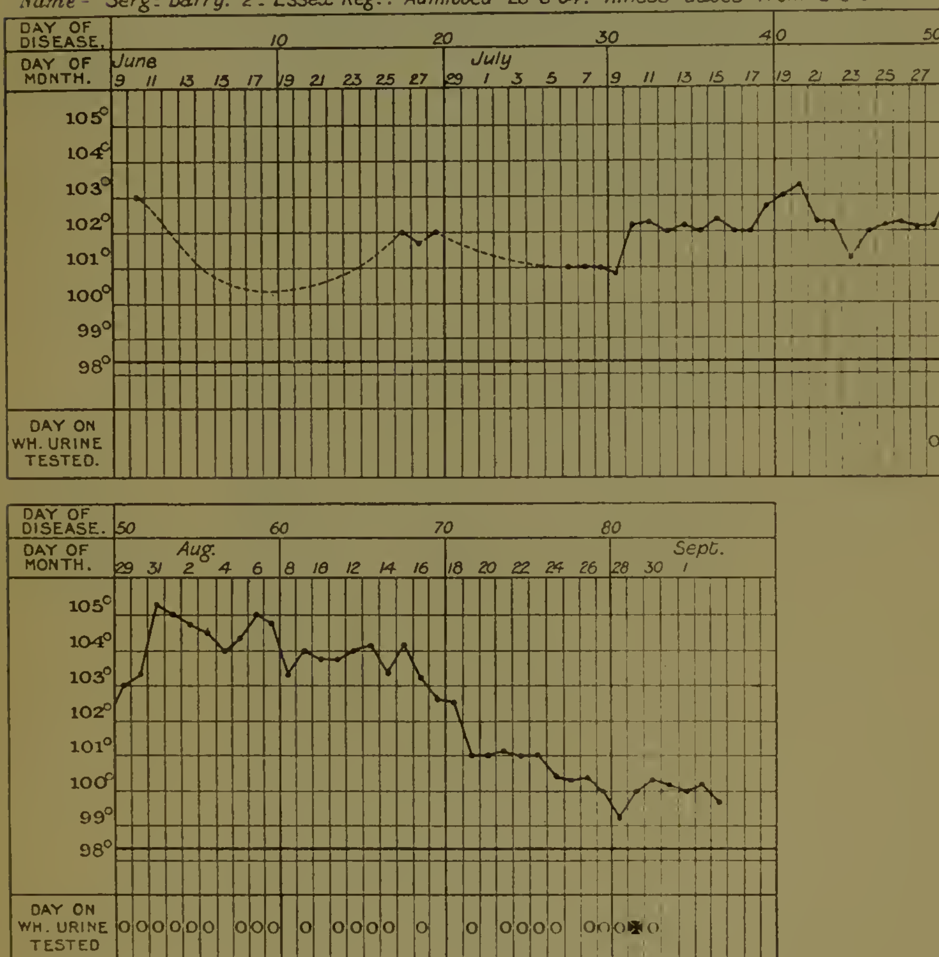
Careful observation of the urines has been made in order to ascertain whether any physical or chemical change is associated with the passage of the *M. melitensis*. All the urines have been free from the general opacity or turbidity, which is associated with Typhoid Bacilluria. A little deposit of mucus has been observed, and a portion of this when plated out has always given more colonies than the clear portion of the urine treated in the same manner. On three occasions a trace of albumen was noticed, but up to the present no physical or chemical change common to all the urines and indicating the passage of the *M. melitensis* has been observed.

Table A shows the number of micrococci per cubic centimetre obtained from each sample of urine, and indicates the dates when the isolation was effected. It will be noticed that the numbers of micrococci excreted are small as compared with the figures recorded by several observers during the bacilluria of typhoid fever. It is possible that the figures given in the table do not represent the actual numbers passed in every case, and that many colonies escaped observation owing to their being crowded out by other microbes. At the same time many of the plates, notably those of Sergeant Pudney and Private Lawson, were nearly pure cultures of *M. melitensis*, and as all the colonies which appeared were perfectly discrete, and there was ample room in the plates for other colonies to develop had they been present, it does not seem probable that the numbers passed greatly exceeded the maximum figures recorded.


Name - Pt. Markham. 2nd Essex Regt. Admitted 13-7-04.
DAY OF DISEASE
10
20
30
40
50
DAY OF MONTH
July
12 14 16 18 20 22 24 26 28 30
Aug.
1 3 5 7 9 11 13 15 17 19 21 23 25 27 29
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED
DAY OF DISEASE
50
60
70
80
DAY OF MONTH
Sept.
31 2 4 6 8 10 12 14 16 18 20 22 24 26 28
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED


Name - Pt. Morten. 2nd Essex Regt Admitted 13-7-04. Illness began on 23rd June.
DAY OF DISEASE
DAY OF MONTH
June
July
Aug.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED
Sept.

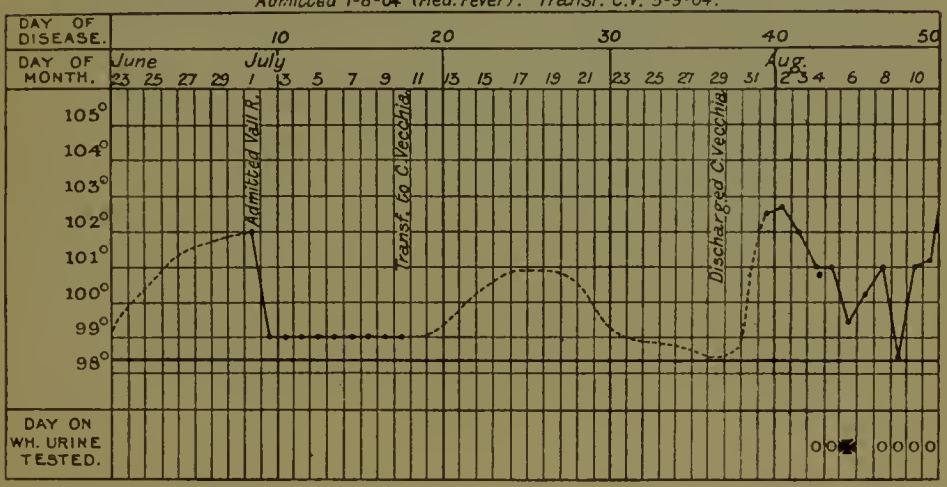
Name - Sergt. Barry. 2nd Essex Regt. Admitted 26-6-04. Illness dates from 9-6-04.

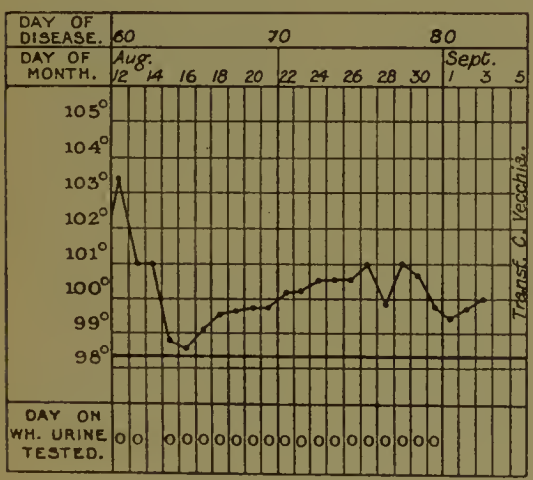


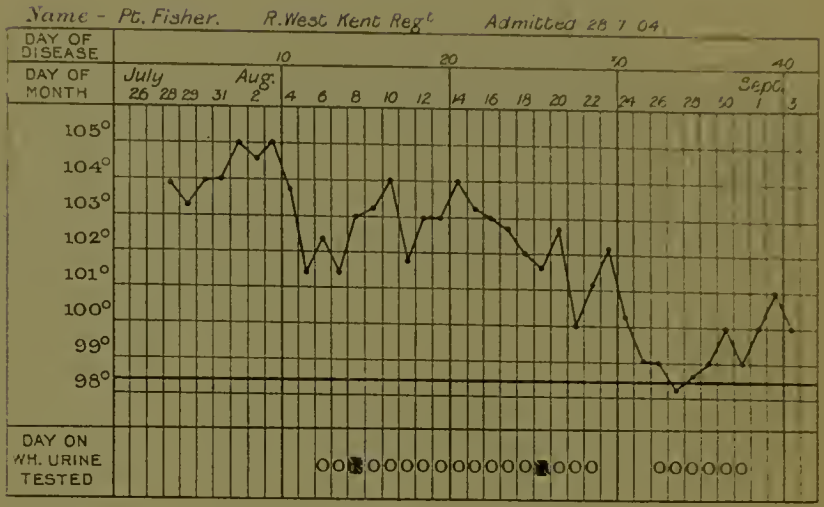
Name- Qtr. Mstr. Sergt. Lawson. A.O.C. att. 2nd Essex Reg. Admitted 15-7-04
DAY OF DISEASE
DAY OF MONTH
July
Aug.
Sept.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED

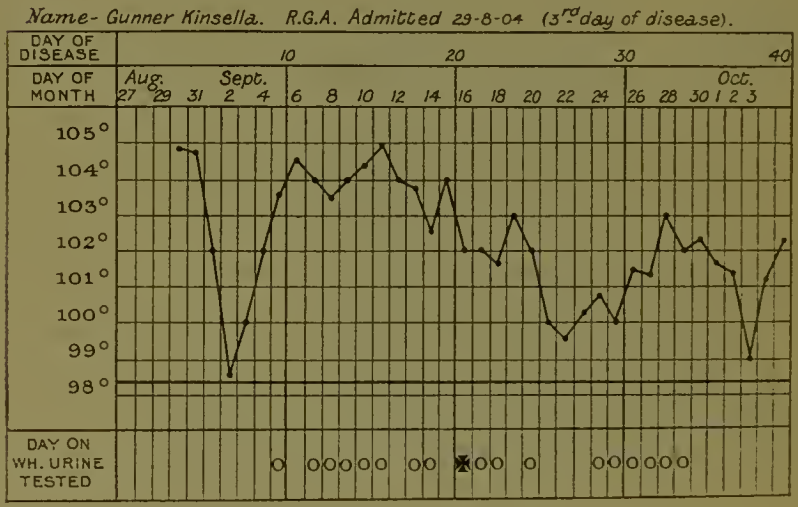
Name- Lc. Sergt. Howe. 2nd Essex Regt.
Admitted 20-6-04, Died 6.30, 6-7-04.
DAY OF DISEASE
DAY OF MONTH
June
July
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED

Name – Boy Bellfield. R. West Kent Regt. Admitted S.C.F. (??) 1-7-04. Transf. C.V. 10-7-04. Admitted 1-8-04 (Med. Fever). Transf. C.V. 5-9-04.

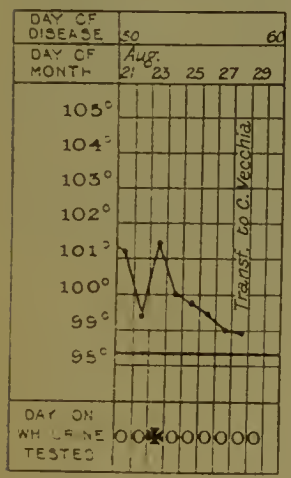


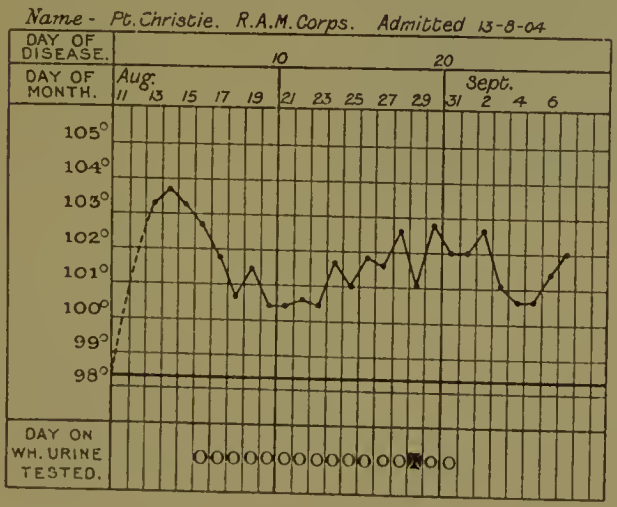


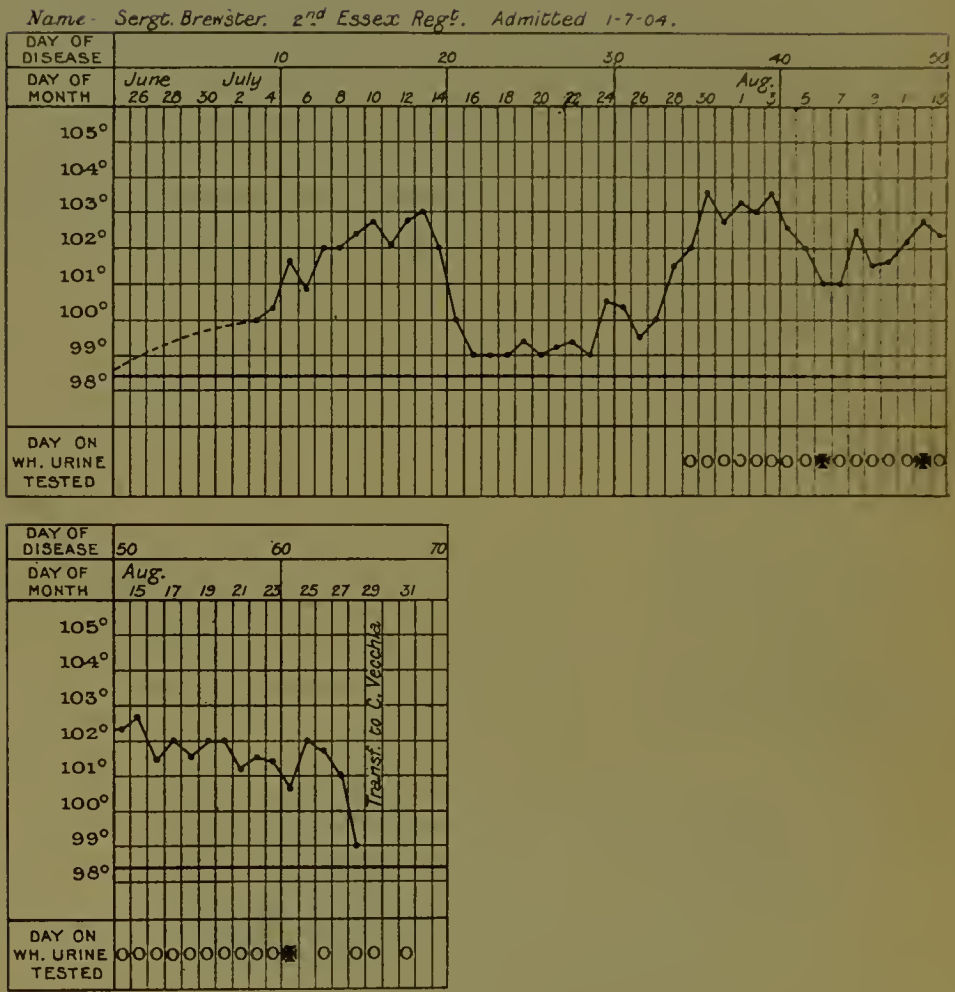

Name - Pt. Fisher. R.West Kent Regt Admitted 28 7 04
DAY OF DISEASE
DAY OF MONTH
July Aug. Sept.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED

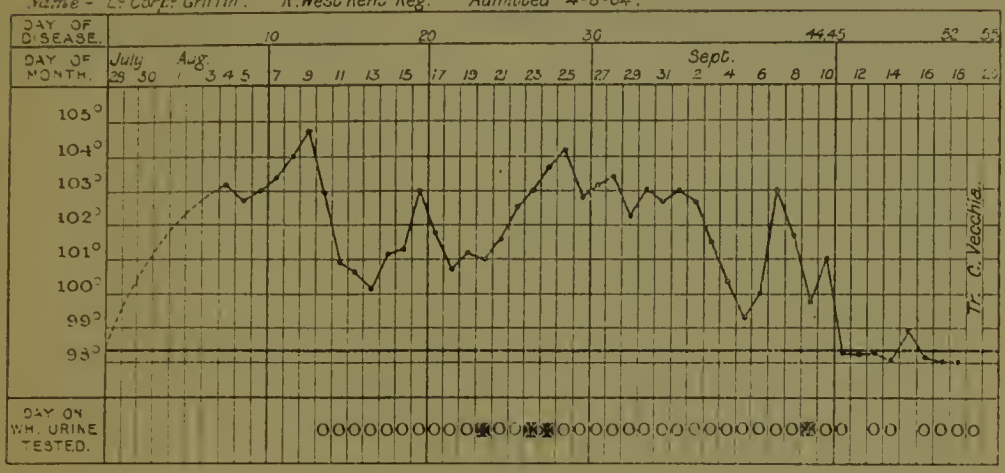

Name- Gunner Kinsella. R.G.A. Admitted 29-8-04 (3rd day of disease).
DAY OF DISEASE
DAY OF MONTH
Aug. Sept. Oct.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED

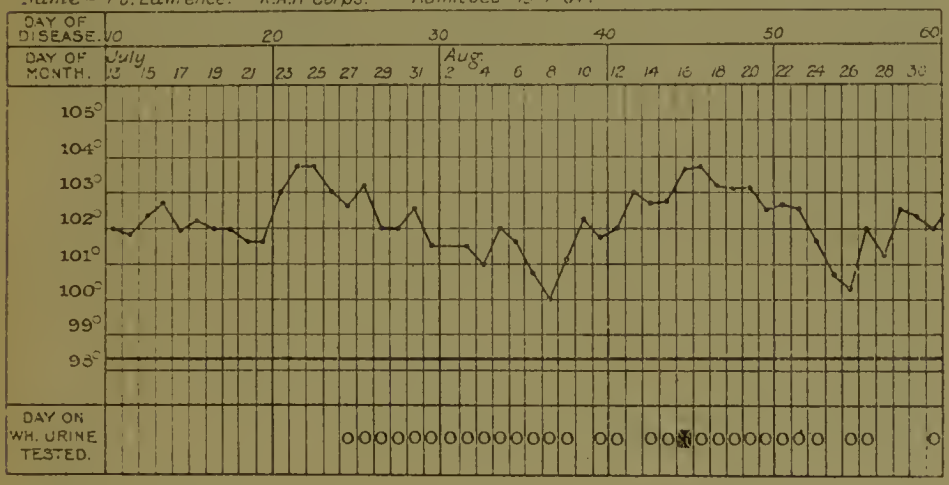

Name - Sergt. Pudney. 2nd Essex Regt.. Admitted 6-7-04. Transferred convalescent 29-8-04
DAY OF DISEASE
DAY OF MONTH
July
Aug.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED

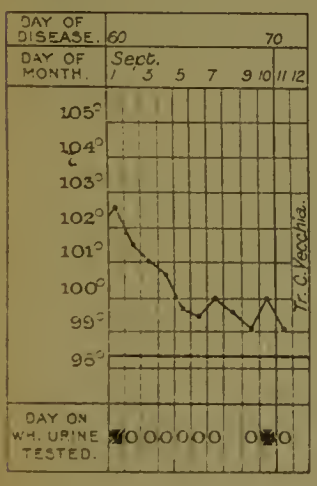

DAY OF DISEASE
DAY OF MONTH
Aug.
105°
104°
103°
102°
101°
100°
99°
95°
Transf. to C. Vecchia
DAY ON WH. URINE TESTED


Name - Pt. Christie. R.A.M.Corps. Admitted 13-8-04
DAY OF DISEASE.
DAY OF MONTH.
Aug.
Sept.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED.


Name - Sergt. Brewster. 2nd Essex Regt. Admitted 1-7-04.
DAY OF DISEASE
DAY OF MONTH
June
July
Aug.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED
Transf. to C. Vecchia.


Name - Lt Capt Griffin. R.West Kent Reg. Admitted 4-8-04.
DAY OF DISEASE.
DAY OF MONTH.
July
Aug.
Sept.
105°
104°
103°
102°
101°
100°
99°
98°
Tr. C. Vecchia.
DAY ON WH. URINE TESTED.


Name - Pt. Lawrence. R.A.M Corps. Admitted 15-7-04.
DAY OF DISEASE.
DAY OF MONTH.
July
Aug.
105°
104°
103°
102°
101°
100°
99°
98°
DAY ON WH. URINE TESTED.


DAY OF DISEASE.
DAY OF MONTH.
Sept.
105°
104°
103°
102°
101°
100°
99°
98°
Tr. C. Vecchia.
DAY ON WH. URINE TESTED.

Up to the present 520 samples of urine have been examined, representing the study of more than 1000 plates.

2. *Examination of Faces.*

Having succeeded in isolating the *M. melitensis* from the urine of Mediterranean fever cases, attempts were now made to detect the microbe in the fæces of these patients. Unfortunately, most of the cases suffered from constipation, and the bowels only acted after the administration of an enema. A few patients suffered from diarrhœa for a short time, and the opportunity was taken of investigating these stools.

The great difficulty to contend with in the study of fæces is caused by the presence of the rapidly growing *B. coli* in large numbers. The enrichment method, which failed with the urine, appeared to be even less likely to yield satisfactory results with fæces. A few trials were made of planting out some of the stools in broth and then, after incubating for four days at 37° C., plating out the growths on glucose-litmus-nutrose-agar plates. The results were highly unsatisfactory: the *B. coli* and its allies converted the plates into a strongly acid medium, on which the *M. melitensis* would not grow. Evidently a medium on which the *B. coli* could not develop, would prove of great assistance in isolating the *M. melitensis* from stools. E. Roth in the *Archiv f. Hygiene* of March 3, 1904, reported that the development of the *B. coli* was arrested in a medium containing 60 per cent. of a solution containing $\frac{1}{100}$th of caffeine. For greater security against the development of *B. coli* he recommended the proportion of caffeine to be increased to 115 per cent. of the $\frac{1}{100}$th solution. Ficker and Hoffmann in the same number of the *Archiv f. Hygiene* also attested the value of caffeine in arresting the development of *B. coli*; they used 5 grammes of caffeine per litre of fluid. Courmont and Lacomme also wrote on caffeine in bacteriology in the March number of the *Journal of Physiology and Pathology*, 1904. They stated that when caffeine was added to broth to the extent of 1 per cent., the development of *B. coli* was prevented. In view of these statements, experiments were made to test the viability of the *M. melitensis* in caffeinised media. Broth tubes were prepared containing 0·5 per cent., 0·75 per cent., and 1 per cent. of caffeine. Each tube was inoculated with a small loopful of an agar growth, derived from the spleen of Sergeant Howe. The results obtained were as follows:—

(1). 5.8.04, 0·5 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.

(2). 5.8.04, 0·75 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.

(3). 5.8.04, 1 per cent. Caffeine broth, inoculated with *M. melitensis* spleen culture of man, incubated at 37° C.

(1). 8.8.04, 0·5 per cent. Good growth. Planted out on agar and *M. melitensis* recovered.

(2). 8.8.04, 0·75 per cent. No growth.
(3). 8.8.04, 1 „ „
(2). 9.8.04, 0·75 „ „
(3). 9.8.04, 1 „ „
(2). 10.8.04, 0·75 „ „
(3). 10.8.04, 1 „ „
(2). 11.8.04, 0·75 „ „
(3). 11.8.04, 1 „ „
(2). 12.8.04, 0·75 „ „
(3). 12.8.04, 1 „ „
(2). 15.8.04, 0·75 „ „
(3). 15.8.04, 1 „ „

(2). 18.8.04, 0·75 per cent. No growth. Planted out on agar slopes. No growths appeared.

(3). 18.8.04, 1 per cent. No growth. Planted out on agar slopes. No growths appeared.

Result.—*M. melitensis* derived from the spleen of man does not appear to develop in media containing more than 0·5 per cent. of caffeine.

Courmont and Lacomme having stated in their paper that cultures of *B. typhosus* from urine were more resistant to the action of caffeine than cultures derived from the blood, experiments were made to see if the same held good for cultures of *M. melitensis*. Accordingly, batches of the same broth used in the previous experiments were inoculated with an agar culture obtained from Sergeant Pudney's urine; the tubes were incubated at 37° C.

The results obtained were as follows :—

(1). 5.8.04, 0·5 per cent. Caffeine broth, inoculated with culture from urine.

(2). 5.8.04, 0·75 per cent. Caffeine broth, inoculated with culture from urine.

(3). 5.8.04, 1 per cent. Caffeine broth, inoculated with culture from urine.

(1). 8.8.04, 0·5 per cent. Good growth. Planted on agar. *M. melitensis* recovered.

(2). 8.8.04, 0·75 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

(3). 8.8.04, 1 per cent. Very feeble growth. Planted on agar. *M. melitensis* recovered.

Result.—The *M. melitensis* derived from urine is able to grow, but only feebly, in broth containing 0·75 and 1 per cent. of caffeine.

A culture of *B. coli* isolated from the stool of a Mediterranean fever case was next tested as to its growth in caffeinised broth. The results obtained were as follows :—

(1). 16.8.04, 0·5 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(2). 16.8.04, 0·75 per cent. Caffeine broth inoculated with *B. coli* from stool of Mediterranean fever case.

(3). 16.8.04, 1 per cent. Caffeine broth, inoculated with *B. coli* from stool of Mediterranean fever case.

(1). 17.8.04, 0·5 per cent. Good growth. Planted on agar. *B. coli* recovered.

(2). 17.8.04, 0·75 per cent. No growth.

(3). 17.8.04, 1 ,, ,,

(2). 18.8.04, 0·75 ,, ,,

(3). 18.8.04, 1 ,, ,,

(2). 19.8.04, 0·75 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

(3). 19.8.04, 1 per cent. Feeble growth. Planted on agar. A few colonies of *B. coli* appeared.

Result.—Caffeine in the proportion of 0·75 and 1 per cent. appeared to have a distinct restraining influence on the growth of *B. coli*.

An emulsion of one loop of *B. coli* and one loop of *M. melitensis*, from a urine culture, was now thoroughly mixed and then plated out on 0·75 per cent. caffeine-glucose-nutrose-litmus-agar. As a result a few colonies of *B. coli* appeared in 48 hours, but no signs of the *M. melitensis* were observed even after 6 days' incubation at 37° C.; evidently the use of media containing more than 0·50 per cent. of caffeine would be attended with considerable risk of arresting the growth of the *M. melitensis*.

A batch of plates, containing 0·5 per cent. of caffeine in addition to the usual glucose-nutrose-litmus-agar, was now prepared. An emulsion of a stool from a Mediterranean fever case was plated out, and as a control the same emulsion in the same quantities was plated on the ordinary glucose-nutrose-litmus-agar. After 48 hours' incubation at 37° C., there was no appreciable difference between the plates, so the use of caffeine was abandoned in this investigation. The technique has consisted in adding loopfuls of the fluid stools, the number of loops depending on the fluidity of each stool, to either sterile salt solution or broth until a slightly opalescent mixture was produced. Loopfuls of the mixture were then stroked concentrically or diffused by means of a "platinum spreader" over the surface of glucose-litmus-nutrose-agar, solidified in Petri dishes. The plates were then placed with the covers downwards in the 37° C. incubator. After 4 and 5 days' incubation the resulting colonies were examined in a hanging drop; if anything like the morphology of *M. melitensis* appeared, the cover-glass was removed, and a loopful of the specific serum, diluted 1—10, added. Many of the streptococci occurring in stools bear a superficial resemblance to the *M. melitensis*: still, as a rule,

Table showing the Days of Disease on which the Stools were Examined, and the Number of Plates made.

No. of case.	Name.	Days of Disease.																																														Total.
		11	12	13	15	16	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	42	43	46	48	49	50	51	52	53	55	56	57	59	60	62	76	77	104	
1	Barry																																					6							12	4	14	26
2	Eldred														4	10																																14
3	Francis							3	2																																							6
4	Vince						5																								9				4													18
5	Moore													5																																		5
6	Brewster																													5																		5
7	Corporal Jones																																						4	3	4							11
8	Griffin				4			8	9	4		4		21		4						16																										70
9	Mays																												4																			4
10	Fisher									8	19	3	6	16	8				16													15	20	15			10				20			12				168
11	Christie							14				21						15	14	18	11		10	10																								113
12	Lawrence																																											8				8
13	Hurrell											24		21	30		15																		14													104
14	Vince																																			14												14
15	Curry									11																																						11
16	Campbell															22																																22
17	Ingram																																															15
18	Groom													20	12	12																																44
19	Gane											12																																				12
20	Silcox																								10	12	10	10	20	14		12																88
21	Private Jones			11						14																																						25
22	Silburn		10			14																																										24
23	Smith													14		12	12	22	12	16																												88
24	Martin								13													12																										25
25	Iggo	12																																														12
26	Rowlands																																									12	12					24
27	Rantiome												14																																			14
28	Kinsella																		16																													16
29	Anthony						14	16																																								30
	No. of plates...	12	10	11	4	14	19	41	25	37	19	64	20	97	54	60	27	37	58	34	11	28	10	10	10	12	10	10	24	19	9	27	20	15	18	14	10	6	4	3	24	12	12	20	12	4	14	1026

the colonies have a faint opacity and sometimes a reddish tinge which enables them to be at once distinguished from the *M. melitensis*. In any case of doubt the addition of the specific serum enabled a diagnosis to be made. The attached table shows the number of stools examined and the results up to the present time. It will be seen that 1026 plates made from eighty-six stools have been studied, but with a negative result.

Examination of Stools of Mediterranean Fever Cases.

Name.	Dates.	Number of plates.	Day of disease.	Result.
1. Barry	31.7.04	6	53	*M. melitensis* not isolated.
2. „	23.8.04	12	76	„ „
3. „	24.8.04	4	77	„ „
4. Eldred	27.7.04	10	27	„ „
5. „	26.7.04	4	26	„ „
6. Franeis	17.7.04	3	19	„ „
7. „	18.7.04	3	20	„ „
8. Vince	23.7 04	5	18	„ „
9. „	17.8.04	9	43	„ „
10. „	24.8.04	4	50	„ „
11. Moore	25.7.04	5	25	„ „
12. Brewster	5.8.04	5	42	„ „
13. Jones	7.8.04	4	55	„ „
14. „	8.8.04	3	56	„ „
15. „	9.8.04	4	57	„ „
16. Griffin	11.8.04	4	15	„ „
17. „	15.8.04	8	19	„ „
18. „	16.8.04	9	20	„ „
19. „	17.8.04	4	21	„ „
20. „	19.8.04	4	23	„ „
21. „	21.8.04	21	25	„ „
22. „	23.8.04	4	27	„ „
23. Mays.....	12.8.04	4	40	„ „
24. Fisher.....	14.8.04	8	21	„ „
25. „	15.8.04	19	22	„ „
26. „	16.8.04	3	23	„ „
27. „	17.8.04	6	24	„ „
28. „	18.8.04	16	25	„ „
29. „	19.8.04	8	26	„ „
30. Christie	2.9.04	21	23	„ „
31. Lawrence.....	2.9.04	8	62	„ „
32. Hurrell.....	23.8.04	24	23	„ „
33. Fisher.....	23.8.04	16	30	„ „
34. Hurrell.....	25.8.04	21	25	„ „
35. Vince	25.8.04	14	51	„ „
36. Hurrell.....	26.8.04	30	26	„ „
37. Curry	27.8.04	11	21	„ „
38. Hurrell	28.8.04	15	28	„ „
39. Griffin	28.8.04	16	33	„ „
40. Christie	29.8.04	14	19	„ „
41. Martin	8.9.04	13	20	„ „
42. Christie	8.9.04	15	29	„ „
43. Fisher.....	8.9.04	15	46	„ „
44. Campbell.....	9.9.04	22	27	„ „
45. Christie.....	9.9.04	14	30	„ „

Examination of Stools of Mediterranean Fever Cases—*contd.*

Name.	Dates.	Number of plates.	Day of disease.	Result.
46. Ingram.....	9.9.04	15	—	*M. melitensis* not isolated.
47. Groom.....	10.9.04	20	25	,, ,,
48. Fisher.....	10.9.04	20	48	,, ,,
49. Christie.....	10.9.04	18	31	,, ,,
50. Groom.....	11.9.04	12	26	,, ,,
51. Christie.....	11.9.04	11	32	,, ,,
52. Fisher.....	11.9.04	15	49	,, ,,
53. Groom.....	12.9.04	12	27	,, ,,
54. Gane.....	12.9.04	12	23	,, ,,
55. Christie	13.9.04	10	34	,, ,,
56. Silcocks.....	13.9.04	10	36	,, ,,
57. Jones.....	13.9.04	11	13	,, ,,
58. Fisher.....	14.9.04	10	52	,, ,,
59. Christie.....	14.9.04	10	35	,, ,,
60. Silcocks.....	14.9.04	12	37	,, ,,
61. ,, ...	15.9.04	10	38	,, ,,
62. ,, ...	16.9.04	10	39	,, ,,
63. Silburn.....	16.9.04	10	12	,, ,,
64. Silcocks.....	17.9.04	20	40	,, ,,
65. Hurrell.....	19.9.04	14	50	,, ,,
66. Silcocks.....	19.9.04	14	42	,, ,,
67. Fisher.....	19.9.04	20	57	,, ,,
68. Barry.....	20.9.04	14	104	,, ,,
69. Smith.....	20.9.04	14	25	,, ,,
70. Silburn.....	20.9.04	14	16	,, ,,
71. Jones.....	21.9.04	14	21	,, ,,
72. Martin.....	21.9.04	12	33	,, ,,
73. Iggo.....	21.9.04	12	11	,, ,,
74. Rowlands.....	22.9.04	12	59	,, ,,
75. Smith.....	22.9.04	12	27	,, ,,
76. Rowlands.....	23.9.04	12	60	,, ,,
77. Smith.....	23.9.04	12	28	,, ,,
78. Silcocks.....	23.9.04	12	46	,, ,,
79. Fisher.....	24.9.04	12	62	,, ,,
80. Smith.....	24.9.04	22	29	,, ,,
81. Rantiome.....	24.9.04	14	24	,, ,,
82. Kinsella.....	25.9.04	16	30	,, ,,
83. Anthony.....	25.9.04	14	18	,, ,,
84. Smith.....	25.9.04	12	30	,, ,,
85. Anthony.....	26.9.04	16	19	,, ,,
86. Smith.....	26.9.04	16	31	,, ,,

3. *Examination of Sweat.*

Critical perspirations, which are very characteristic of Mediterranean fever, have been examined at various periods of the disease, but the *M. melitensis* has not yet been isolated. The following examinations have been made:—

Experiment I.—On June 22, 1904, P . . . was noticed to be sweating profusely. The sweat was soaked up by means of sterile swabs which were then planted out in broth and rubbed over nutrose-agar plates

The tubes and plates were incubated at 37° C. On June 25, 1904, all the broth tubes showed a growth which was plated on nutrose-agar. The primary and secondary agar plates were carefully examined from time to time, but no signs of the *M. melitensis* could be discovered.

Experiment II.—At 8.30 P.M. on June 22, 1904, P . . . was again sweating profusely; swabs were treated as above, but the *M. melitensis* did not appear in the plates.

Experiment III.—At midnight on June 22, 1904, profuse sweats occurred in the same case, and the procedure detailed under Experiment I was followed. The *M. melitensis* was not isolated.

Experiment IV.—In the broth tubes, prepared as above, many contaminations were observed, which often rapidly overgrew the plates and so possibly prevented the *M. melitensis* from developing. In order to get rid of these extraneous organisms as far as possible the skin of P . . . was carefully washed with carbolic acid and ether, and a sterile pad covered by a sterile watch glass was bandaged on the right arm. On June 27, 1904, a critical sweat occurred, the pad was removed and planted out in broth; a growth occurred on June 29, 1904, which was found to consist of large Gram-staining cocci; no signs of the *M. melitensis* were discovered.

Experiment V.—On June 28, 1904, the procedure detailed under Experiment IV was followed in the case of H . . . large Gram-staining cocci again appeared.

Experiment VI.—On June 27, 1904, the same procedure was followed in the case of K . . . large and small Gram-staining cocci were isolated, but the *M. melitensis* did not appear.

Experiment VII.—On June 29, 1904, saturated pads obtained from P . . . were examined; the broth tubes remained absolutely sterile, although the incubation was continued for 10 days.

Experiment VIII.—On June 29, 1904, pads from Wildbore were planted out in broth. No growth resulted.

Experiment IX.—On June 29, 1904, pads from Wilson were planted out in broth. A growth occurred which, when plated, was found to give rise to large colonies, consisting of large cocci staining with Gram.

Experiment X.—On June 30, 1904, pads from Kelly were planted out in broth. No growth resulted.

It might be thought that the failure to obtain a growth recorded under Experiments VII, VIII, and X was possibly due to the presence in the swabs of carbolic acid, which, when transferred to the broth tubes, might inhibit the growth of the *M. melitensis*. In order to ascertain whether this was the case, sterile broth tubes, obtained in the manner detailed, were inoculated with *M. melitensis*. A typical growth resulted, showing that the failure to obtain a growth was not due to the presence of the disinfectant.

Experiment XI. Monkey No. 74.

To determine if the Injection of Sweat, from Malta Fever Patients, into a Monkey will give rise to the specific Fever.

The monkey arrived on August 29, 1904, and was taken at once to the roof of the Station Hospital, Valletta.

September 12, 1904. Skin scrapings were taken from the arms and axillæ of Private Lawrence, and ground up with normal salt solution. The resulting emulsion was injected subcutaneously into Monkey No. 74.

September 17, 1904. The blood was examined; the serum in a low dilution appeared to have a tendency to agglutinate the *M. melitensis.*

September 23, 1904. The blood was again examined, but the serum, diluted 1—10, did not show any signs of agglutinating the *M. melitensis*, even after waiting 1 hour.

September 25, 1904. Skin scrapings made into an emulsion with salt solution, were again injected.

September 27, 1904. Skin scrapings, treated as before, were injected.

September 28, 1904. The blood was examined, but the serum gave no reaction with the *M. melitensis.*

October 24, 1904. Staff-Surgeon Shaw continued the experiment up to this date. An agglutinative reaction was obtained with the serum, diluted 1—40, twenty-two days after the first injection.

The final result will be found in Dr. Shaw's experiments.

Experiment XII.

To determine if the Injection of Bacteria Free Sweat, derived from Malta Fever Patients, causes the Development of Agglutinins in the Blood of a Monkey.

Monkey No. 61A arrived in the laboratory on September 9, 1904. On September 15, 1904, and September 21, 1904, the serum was added in a low dilution to an emulsion of the *M. melitensis*; no trace of agglutination was observed.

September 22, 1904. Skin scrapings were taken from the arms and axillæ of Privates Kinsella and Silburn, who were suffering from Mediterranean Fever, and ground up with normal salt solution so as to form a fine emulsion. A sterile Berkefeld candle having been inserted into a sterile test-tube, the emulsion was filtered so as to remove all bacteria. The filtrate was then injected subcutaneously into Monkey No. 61A.

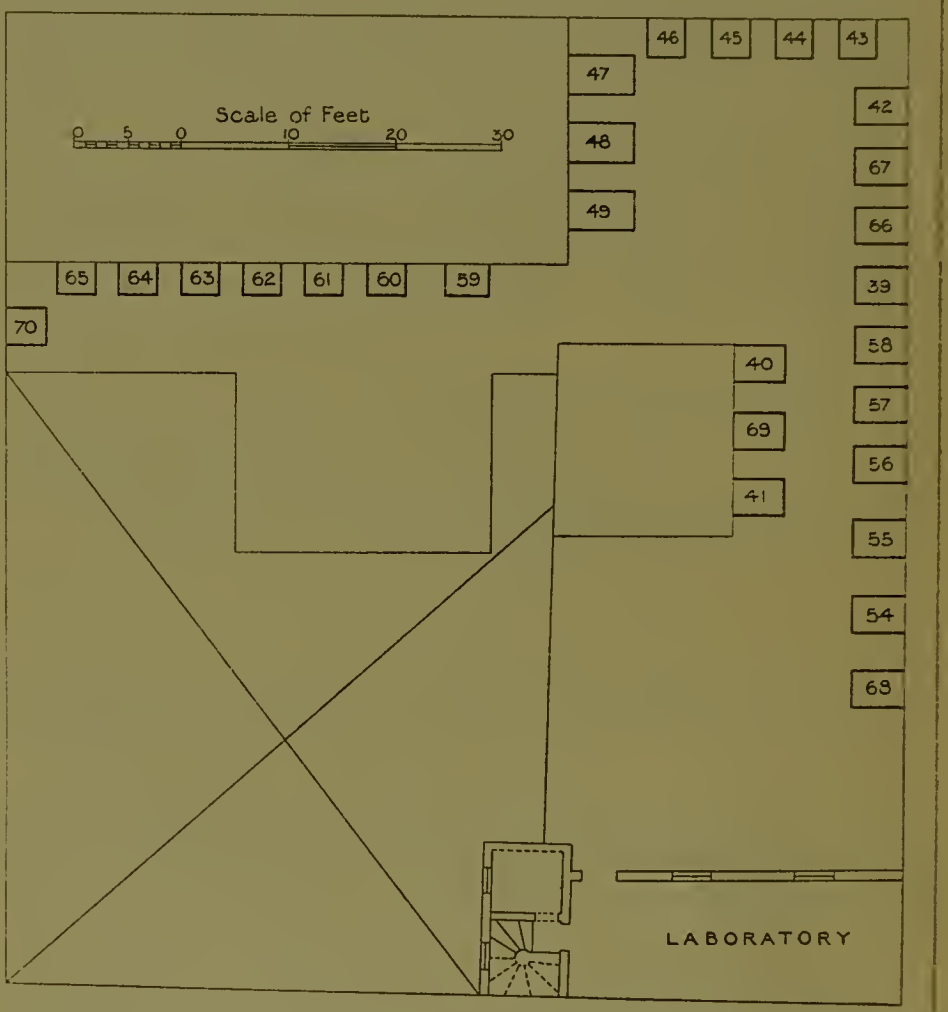
September 24, 1904. Sweat obtained from Privates Smith,

Silcocks, and Kinsella was similarly filtered, and the filtrate injected subcutaneously.

September 26, 1904. The blood was examined, and the serum found to have no action on the *M. melitensis.*

October 24, 1904. Dr. Shaw continued the experiment up to this date. The blood serum never caused the slightest agglutination of the *M. melitensis.*

Result.—The bacteria free filtrate obtained from the sweat of Malta fever patients does not appear to give rise to agglutinins in the blood of a monkey.



Plan of the Roof where Monkeys were kept, showing Position of the Animals which became naturally infected.

4. *Examination of Expired Air of Malta Fever Patients.*

In order to ascertain the presence of the *M. melitensis* in the expired air of Malta fever patients, a test-tube was fitted with an indiarubber

bung through which passed two glass tubes: one, attached to a mouth-piece, reached to the bottom of the test-tube and the other the exit tube, just passed through the bung. The test-tube was half-filled with nutrient broth and the whole apparatus then sterilised in the autoclave.

The patient under examination was directed to force expired air through the broth at frequent intervals throughout the day. The indiarubber bung, with glass tubes, was then removed, and the test-tube, being plugged with sterile cotton wool, was incubated at 37° C. After four days' incubation the broth was plated on nutrose-glucose-litmus-agar plates, and likely colonies fished and tested in the usual manner.

Case 1.—Private Markham breathed through one of these tubes on the 12.9.04; the tube was then incubated at 37° C. Four days later there was no sign of growth, but on the 19.9.04 a slight opalescence was noted. The broth was then plated on nutrose-glucose-litmus-agar. The plates were incubated for seven days, but no colonies of the *M. melitensis* appeared.

Case 2.—Private Lawrence breathed through a tube on the 12.9.04. On the 16.9.04 a marked growth appeared. A portion of the broth was plated as above, and the remainder of the growth injected into Monkey No. 73. After seven days' incubation no signs of *M. melitensis* could be discovered in the plates.

Case 3.—Private Markham again breathed through a tube on the 14.9.04. The tube was treated as before, and a slight growth was noticed on the 21.9.04. The growth was then plated, but no colonies of the *M. melitensis* appeared.

Case 4.—Private Lawrence breathed through a tube on the 14.9.04. On the 21.9.04 a slight growth appeared, which was then plated as before. No colonies of the *M. melitensis* were seen in the plates.

Case 5.—Private Kinsella breathed through a tube on the 17.9.04. On the 26.9.04 a slight growth appeared, but no colonies of *M. melitensis* were discovered in the plates made with the opalescent broth.

Case 6.—Private Silburn breathed through a tube on the 17.9.04. After twenty-four hours' incubation, the broth, being distinctly turbid, was plated in the usual manner, and incubation of the tube continued. Four days later a portion of the growth in the test-tube was plated out and the remainder of the growth injected into Monkey No. 73. No signs of the *M. melitensis* were discovered in the plates after prolonged incubation at 37° C.

Case 7.—Private Kinsella again breathed through a tube on the 20.9.04. No growth appeared in the broth, though incubation was continued for fourteen days.

Case 8.—Private Silburn breathed through a tube on the 20.9.04. A marked growth, having a putrefactive odour, appeared on the 24.9.04. This was then plated out as usual, but no colonies of the *M. melitensis* were discovered.

Case 9.—Private Silburn again breathed through a tube on the 23.9.04. The growth which appeared after incubation was treated in the usual manner, but no colonies of *M. melitensis* were isolated.

Case 10.—Private Tripp breathed through a tube on the 23.9.04. The tube was plated as before, but the *M. melitensis* was not isolated.

Case 11.—Private Anthony breathed through a tube on the 23.9.04. After the usual incubation the resulting growth was plated out, but with a negative result.

Case 12.—Private Rivers breathed through a tube on the 23.9.04. After the usual treatment, the *M. melitensis* was not isolated.

Monkey No. 73.

This monkey was reserved for the injection of broth infected by the expired air of Malta fever patients.

The monkey arrived at the laboratory on the 8.9.04. On the 15.9.04 a portion of its blood was removed and the serum, in a low dilution, added to an emulsion of the *M. melitensis*. No traces of agglutination were observed. On the 16.9.04 10 c.c. of broth infected by the breath of Private Lawrence were injected subcutaneously. On the 21.9.04 10 c.c. of broth infected by the breath of Private Silburn were injected. The action of the blood serum on the *M. melitensis* was also tested on this day, but no signs of agglutination were observed. On the 28.9.04 the blood serum was again examined, but no reaction with the *M. melitensis* was observed, though the dilution of the serum was only 1—10.

5. *Examination of Sea-water in the Grand Harbour, Malta.*

Having in view the result obtained when studying the viability of the *M. melitensis* in sea-water, and the fact that sea-water is extensively used for washing the decks of the battleships stationed in the Grand Harbour, it appeared desirable to ascertain whether the *M. melitensis* could be discovered in sea-water taken from this locality.

Studies of sea-water, when unsterilised and grossly infected with the *M. melitensis*, soon showed that the specific microbe could not be isolated, by ordinary bacteriological methods, a few days after the infection, owing to the saprophytic organisms overgrowing the colonies of the *M. melitensis*. Accordingly, it was decided to filter the sea-water through a sterile Berkefeld candle, and after washing the deposit with tap-water, to suspend it in 10 c.c. of tap-water, and inject the whole subcutaneously into a monkey.

On the 9.9.04 600 c.c. of sea-water, taken from the Grand Harbour opposite Fort St. Angelo, were pumped through a Berkefeld candle, and the deposit, having been well washed, was diffused in 10 c.c. of tap-water and injected subcutaneously into Monkey No. 71.

On the 10.9.04, the deposit from 600 c.c. of sea-water, taken from the same place, was injected.

On the 13.9.04, the deposit from 600 c.c. of sea-water, taken as before, was injected.

On the 15.9.04 the same procedure was followed.

On the 17.9.04 the same procedure was followed.

On the 18.9.04 the serum of Monkey No. 71 was added to an emulsion of the *M. melitensis*. No traces of agglutination were observed.

On the 19.9.04 600 c.c. of sea-water, taken off Fort St. Angelo, were again filtered, washed, and injected.

On the 21.9.04 the same procedure was followed.

On the 23.9.04 the same procedure was followed.

On the 25.9.04 1800 c.c. of sea-water were treated as before and the deposit injected. The serum of the monkey was added to an emulsion of *M. melitensis*, but no reaction was obtained.

On the 27.9.04 1800 c.c. of sea-water were filtered, and the washed deposit injected.

On the 29.9.04 600 c.c. of sea-water were treated as before. There is a small abscess at the site of the inoculation of the 27th.

Dr. Shaw continued this experiment up to October 22; the monkey received the bacteria contained in 30 litres of sea-water, but the blood serum never caused the slightest agglutination of the *M. melitensis*.

Result.—The *M. melitensis* could not be detected in the sea-water of the Grand Harbour.

4.

EXPERIMENTS ON THE MODE OF CONVEYANCE OF THE *MICROCOCCUS MELITENSIS* TO HEALTHY ANIMALS.

By Major W. H. Horrocks, R.A.M.C., Member Mediterranean Fever Commission.

(Received September 17, 1904.)

Experiment I.—Monkey No. 41.

To Determine if the Inhalation of Dust, Infected with M. melitensis, *will give Rise to Mediterranean Fever in Healthy Monkeys.*

July 10, 1904. Monkey placed in cage and infected dust blown round him. Dust in bottle A used for this experiment, infected July 2, 1904.

July 11, 1904. Monkey kept in the cage and dust again blown round him. It was noticed, however, that owing to the moisture condensed on the walls, the dust soon settled, and it was impossible to keep it passing backwards and forwards through the cage. After an hour's interval, the cage was opened and the monkey allowed to come out into the room. Cage was then disinfected and dried.

July 12, 1904. Same procedure as July 10, 1904.
„ 13, „ „ „
„ 14, „ „ „
„ 15, „ „ „
„ 16, „ „ „
„ 18, „ „ „
„ 19, „ „ „
„ 20, „ „ „
„ 21, „ „ „
„ 22, „ Tested blood. No reaction.
„ 23, „ Placed in cage; dust blown as before.
„ 25, „ Placed in cage. The dust (bottle A) all expended.

Planted out one loop in broth to try and determine presence of *M. melitensis*. July 26, 1904, growth planted on agar; no signs of *M. melitensis*.

July 25, 1904. Prepared more dust to-day; dust (Petri dish half full) sterilised, and then inoculated with four agar slopes, third generation from spleen of man, dried over sulphuric acid *in vacuo*.

July 29, 1904. Monkey placed in cage and dust blown as before; dust dried over sulphuric acid employed.

July 31, 1904. Monkey placed in cage and dust blown as before; dust dried over sulphuric acid employed.

Note.—The dust appears to fall very rapidly; only seen on the nostrils. Mouth, as a rule, kept tight shut.

August 1, 1904. The same procedure as on July 29, 1904.

" 2, " " "

August 3, 1904. The same procedure as on July 29, 1904. Planted out soil in broth to see if *M. melitensis* still present; growth August 6, 1904, planted on agar. *M. melitensis* recovered.

August 4, 1904. The same procedure as on July 29, 1904; dust all expended.

August 5, 1904. Fresh dust prepared. Four tubes, second generation, spleen of Howe, incubated 3 days at 37° C., dried 24 hours over sulphuric acid and $CaCl_2$ *in vacuo*. Dust blown in cage. Dust planted out in broth on August 4, 1904, to ascertain presence of *M. melitensis*. August 8, 1904, planted on glucose agar; no *M. melitensis*, isolated; broth probably contaminated. This batch of broth found to be contaminated with *B. mesentericus*.

August 6, 1904. Dust blown in cage as on August 5, 1904. Dust planted out in broth August 9, 1904. Growth planted on agar August 10, 1904; broth contaminated, cause probably as on August 4, 1904.

August 8, 1904. Dust blown as before.

August 9, 1904. Planted out dust in broth (proved by incubation). On August 13, 1904, growth planted on glucose-litmus-agar, *M. melitensis* present.

August 10, 1904. Dust blown as before.

August 16, 1904. Examined blood; serum gave no reaction with *M. melitensis* in a dilution of 1 in 10.

August 26, 1904. Examined blood; serum reacted at once with *M. melitensis* in a dilution of 1 in 20; no reaction 1 in 50.

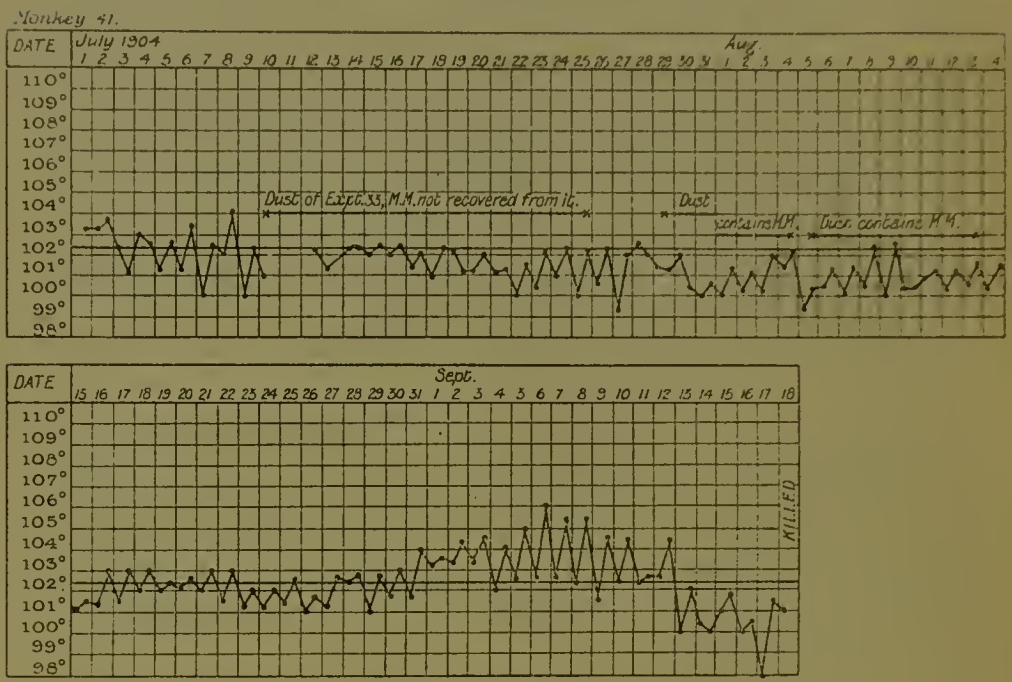
September 6, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—100; no reaction 1—500.

September 15, 1904. Examined blood; serum reaeted at once, visible to naked eye, dilution 1—50; reaction incomplete in a dilution of 1—100.

September 19, 1904. Killed the monkey with chloroform. *Post-mortem* examination: Spleen enlarged, soft, and friable. Liver and kidneys congested. Made cultures from spleen, liver, and kidneys, urine, and heart's blood.

September 23, 1904. *M. melitensis* isolated from spleen of this monkey. Cultures made from liver, kidneys, and heart's blood are sterile.

The following chart represents the course of the *rectal* temperature:



Monkey No. 41.

Note.—The wave of fever did not commence until August 31, though a slight serum reaction was obtained on August 26. The first date on which the dust was known to contain the *M. melitensis* was July 29, consequently the incubation period might have varied from 17 to about 30 days.

Result.—This experiment seems to show that the inhalation or ingestion of infected dust will give rise to the disease.

Experiment II.—Monkey No. 47.

To determine if the Injection of Dust, infected with M. melitensis, *into the Nostrils and Throat will give rise to Mediterranean Fever in Healthy Monkeys.*

July 9, 1904. Injected dry dust containing *Micrococcus melitensis*, 7 days old, into both nostrils of above monkey. (Bottle A of July 2, 1904, used—Experiment 33.)

July 10, 1904. Injection repeated.

„ 11, „ „

„ 12, „ „

July 13, 1904. Injection repeated.

„ 14, „ „

„ 21, „ Examined blood; no reaction with *M. melitensis.*

„ 28, „ „ „ „

„ 29, „ Injected infected dust, dried 2 days over sulphuric acid *in vacuo*, into back of throat; lips covered with a cloth, and tube passed through a wooden gag.

July 30, 1904. Injection repeated as on July 29, 1904.

August 1, „ „ „

„ 2, „ „ „

„ 3, „ „ „

„ 4, „ „ „

„ 5, „ Injection repeated, fresh dust prepared from four agar slopes, spleen Howe, second generation, incubated 3 days at 37° C., then dried for 24 hours over sulphuric acid and calcium chloride *in vacuo.*

August 6, 1904. Injection repeated as on August 5, 1904.

„ 8, „ Injection repeated. The greatest care is being taken to prevent abrasions of the mucous membrane; a wooden gag is inserted between the teeth as before.

August 9, 1904. Examined blood; serum reacts completely to naked eye, dilution 1—40; slight reaction 1—80. No abrasions to be seen in the mouth; on the skin of the lower lip there is a very small abrasion, caused by the gag, but it is unlikely that this was the source of infection, as the lips have been covered as much as possible when the dust was blown.

August 16, 1904. Complete reaction at once 1—100, naked eye; slight reaction 1—300.

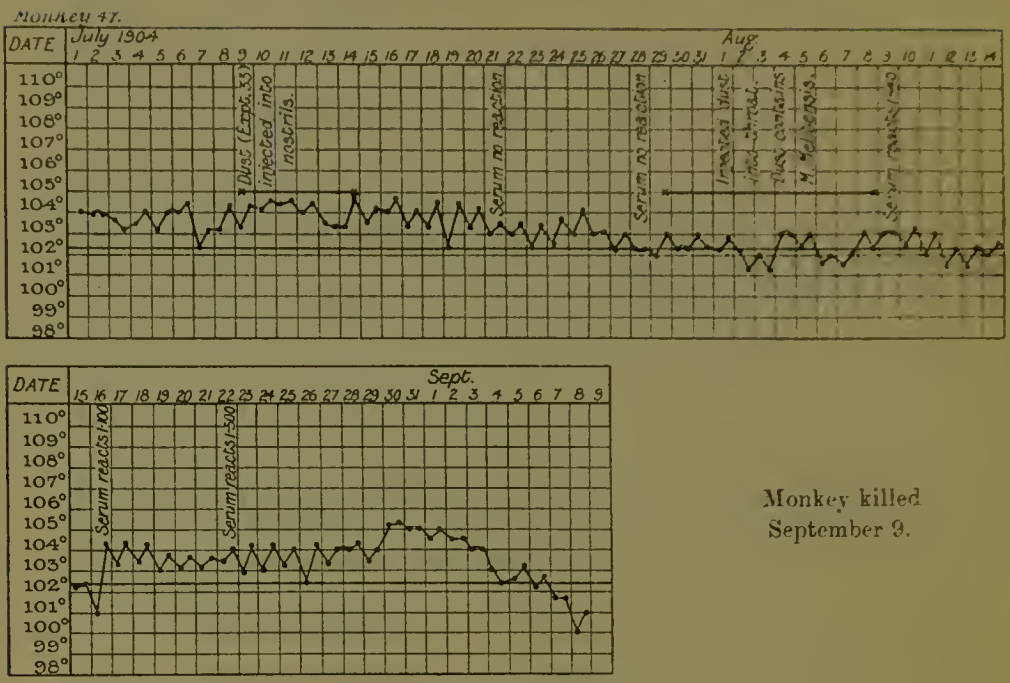
August 22, 1904. Examined blood, complete reaction at once 1—200; complete reaction, visible to naked eye in 10 minutes, dilution 1—500; 1—1000 dilution, *nil.*

September 9, 1904. This monkey has been very ill for some days, and has lost flesh rapidly. Being obviously in a dying state, he was killed with chloroform this morning. *Post-mortem* examination: Spleen enlarged, soft and friable. Kidneys markedly congested. Liver congested. Pericardium contained some fluid. Other viscera healthy

Made cultures from the spleen, kidneys and liver.

The *M. melitensis* was not recovered, as all the cultures proved to be contaminated. The monkey was dying, and a batch of broth, which had not been tested by incubation, had to be used; unfortunately, all the broth tubes were found, on incubation, to be contaminated by *B. mesentericus.*

The following chart represents the course of the temperature:—



Monkey No. 47.

Result.—From this and the last experiment it is evident that the inhalation or swallowing of infected dust will give rise to Mediterranean Fever in monkeys.

Experiment III.—Monkey No. 39.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

This monkey was kept under observation from July 1—10, 1904. It appeared perfectly healthy, and no cuts or abrasions were visible either on the body or in the mouth.

July 10, 1904. The growth from one agar slope, second generation, from spleen of man, and grown for 7 days at 37° C., was mixed with boiled potato, and eaten by the monkey.

July 11, 1904. The growth from one agar slope, as above, but grown for 8 days at 37° C., was mixed with boiled potato and two plums, and eaten by the monkey.

July 12, 1904. The same procedure followed, but the agar slope was 9 days old.

July 13, 1904. As on the 12th; growth 10 days old.

July 14, 1904. The same procedure followed, but a 9 days' old culture from heart's blood of a rabbit was employed.

July 15, 1904. Ten days' old culture, third generation, spleen of man used.

July 16, 1904. The same as on the 15th.

„ 18, „ „ „

„ 19, „ Feeding continued as on July 15, 1904.

„ 20, „ Feeding continued as on July 15, 1904. Examined blood, serum diluted 1—10, gave no reaction with the laboratory strain of *M. melitensis.*

July 21, 1904. Feeding continued. One agar slope, first generation, spleen H——, incubated for 7 days at 37° C., used.

July 22, 1904. Feeding continued. One agar slope, first generation, kidney H——, incubated for 8 days at 37° C., used.

July 23, 1904. The feeding was continued, but I omitted the plums from the mixture, as I found they gave rise to a strongly acid reaction which might inhibit or destroy the *M. melitensis.* One agar slope, first generation, kidney of H——, incubated for 9 days at 37° C., was employed.

July 25, 1904. Half an agar tube of third generation, spleen of H——, was given. The blood was examined for agglutination, but the serum, diluted 1—10, gave no reaction with the *M. melitensis.*

July 26, 1904. Half an agar tube of third generation, spleen of H——, incubated for 4 days at 37° C., was employed.

July 27, 1904. One agar slope, third generation, spleen of H——, incubated for 14 days at 37° C., mixed with potato.

July 28, 1904. One agar slope, fourth generation, spleen of H——, incubated for 5 days at 37° C., mixed with potato.

July 29, 1904. One agar slope, fourth generation, spleen of H——, incubated for 5 days at 37° C., mixed with potato.

July 30, 1904. One agar slope, fifth generation, spleen of H——, incubated for 5 days at 37° C., mixed with potato.

August 1, 1904. One agar slope, fifth generation, spleen of H——, incubated for 5 days at 37° C., mixed with potato.

August 2, 1904. One agar slope, fifth generation, spleen of H——, incubated for 5 days at 37° C., mixed with potato.

August 3, 1904. One agar slope, second generation, spleen of H——, incubated for 3 days at 37° C., mixed with potato. Only a small portion was consumed.

August 4, 1904. One agar slope, fourth generation, spleen of H——, incubated for 5 days at 37° C. Only a small portion was eaten.

August 5, 1904. One agar slope, second generation, from urine of Sergeant P-——, and incubated 10 days at 37° C., mixed with potato.

August 6, 1904. One agar slope, third generation, spleen of H ——, incubated for 72 hours at 37° C., mixed with potato.

August 8, 1904. One agar slope, third generation, spleen of H——, incubated for 6 days at 37° C., mixed with potato.

August 9, 1904. One agar slope, third generation, spleen of H——, incubated for 6 days at 37° C., mixed with potato.

August 10, 1904. One agar slope, third generation, spleen of H——, incubated for 15 days at 37° C., mixed with potato. Examined blood; serum reacts at once, visible to the naked eye, in a dilution of 1—80; under the microscope reaction is seen at once with a dilution of 1—160.

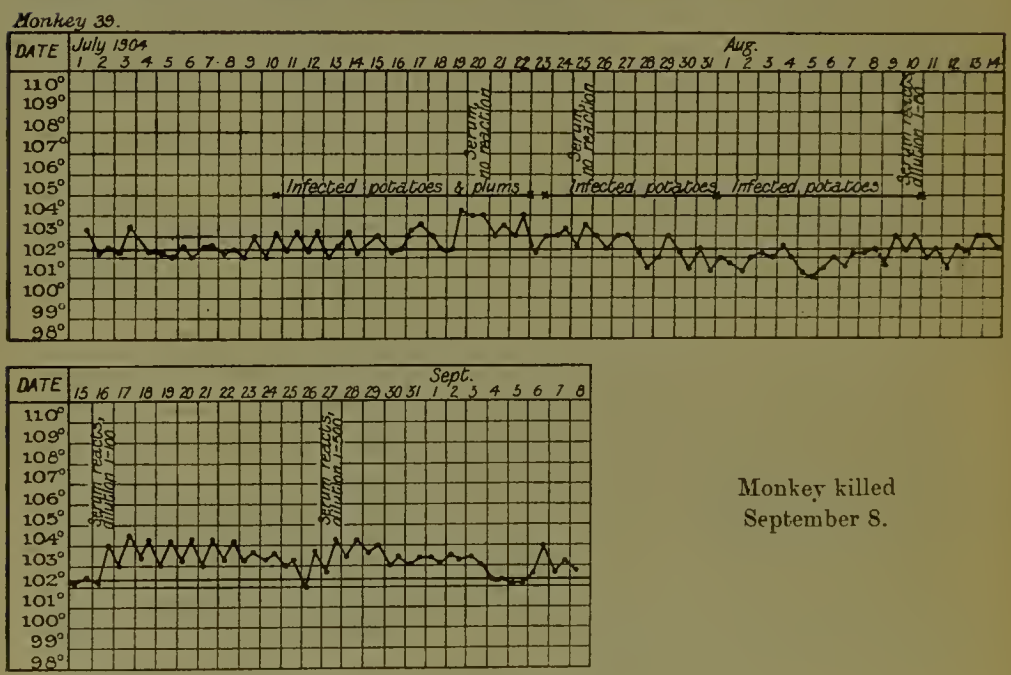
August 16, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. Dilution 1—300 shows a reaction under $\frac{1}{12}$th.

August 27, 1904. Examined blood; serum reacts at once, visible to the naked eye, dilution 1—100. After 5 minutes, dilution 1—500, is visible to the naked eye.

September 8, 1904. Killed the monkey with chloroform. Body well nourished. *Post-mortem.* Spleen enlarged, soft and friable. Kidneys congested Liver congested. Other viscera normal.

September 14, 1904. Recovered *M. melitensis* from the spleen.

The following chart represents the temperature curve :—



Monkey No. 39.

Result.—The absorption of the *M. melitensis* was extremely slow, but the monkey eventually suffered from an acute infection.

Experiment IV.—Monkey No. 40.

To Determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

July 10, 1904. Half of the potato prepared for Monkey No. 39 was given to this monkey. The dose of *M. melitensis* corresponded to one agar slope, as in the case of Monkey No. 39.

July 11, 1904. The same procedure was followed as in Experiment III, Monkey No. 39.

" 12, " " " " "

" 13, " " " " "

" 14, " " " " "

" 15, " " " " "

" 16, " " " " "

" 18, " " " " "

" 19, " " " " "

" 20, " " " " "

" 21, " " " " "

" 22, " " " " "

" 23, " " " " "

" 25, " The same procedure was followed as in Experiment III, Monkey No. 39. Examined blood; serum gave no reaction with *M. melitensis*.

" 26, " The same procedure was followed as in Experiment III, Monkey No. 39.

" 27, " " " " "

" 28, " " " " "

" 29, " " " " "

" 30, " " " " "

August 1, " " " " "

" 2, " " " " "

" 3, " " " " "

" 4, " " " " "

" 5, " " " " "

" 6, " " " " "

" 8, " " " " "

" 9, " " " " "

" 10, " " " " "

" 11, " Examined blood. Complete instantaneous agglutination, visible to the naked eye, dilution 1—30. After standing 5 minutes, dilution 1—100; was also visible to the naked eye.

August 20, 1904. Examined blood. Serum gave a reaction with *M. melitensis* when diluted 1—10, but no result was obtained with higher dilutions.

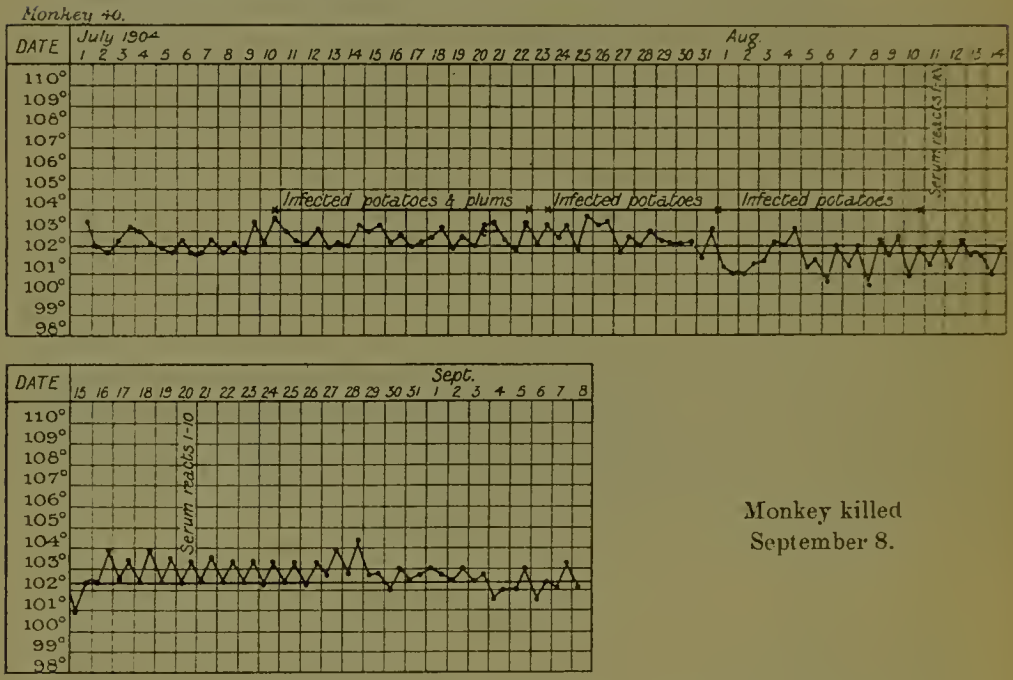
September 8, 1904. Monkey killed by chloroform. *Post-mortem:*

Spleen enlarged, but not so markedly as No. 39; kidneys congested; other viscera apparently healthy. Made cultures from the spleen, liver, and kidneys.

September 16, 1904. *M. melitensis* not recovered from the cultures made at the *post-mortem* examination. All the cultures proved to be sterile.

Note.—It seems probable that, in the case of this monkey, the bacterial infection was never marked, and that the few micrococci absorbed might easily have been destroyed.

The following chart represents the temperature curve:—



Monkey No. 40.

Experiment V.—Monkey No. 66.

To determine if the Ingestion of Infected Food will give rise to Mediterranean Fever in Healthy Monkeys.

August 13, 1904. This monkey is in a box next to Monkey No. 39, and I noticed about a week ago that he ate some of the infected potato provided for No. 39. Examined blood, serum reacts instantaneously, visible to naked eye, dilution 1—100. Visible under $\frac{1}{12}$th after 10 minutes in a dilution of 1—500.

August 18, 1904. Believing this monkey to be healthy, Dr. Zammit, at 6.30 P.M. last evening, injected a small quantity of blood from a Mediterranean Fever patient. In order not to vitiate both experiments the monkey was killed at 11 this morning.

Post-mortem examination :—

Abdomen : Spleen enlarged and congested. Kidney enlarged and congested. Liver congested. Intestines appeared normal.

Thorax : Lungs healthy. Heart appeared dilated.

Cultures made as follows :—

Spleen : (*a*) Planted out in broth and (*aa*) rubbed over an agar slope.
(*b*) Kidney planted out in broth and (*bb*) rubbed over an agar slope.
(*c*) Liver planted out in broth.
(*d*) Heart's blood, planted out in two broth tubes.
(*e*) Urine, planted out in broth.

August 21, 1904. Typical colonies have appeared on the agar slope, made from the spleen ; fished one—it agglutinated at once with serum from Monkey No. 45.

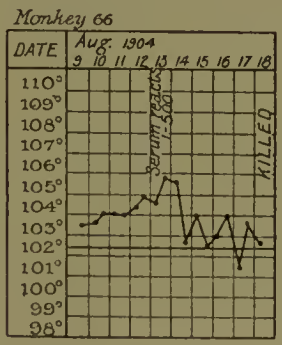
August 22, 1904. Planted out colony from spleen on an agar slope.
„ growth in broth, from heart's blood (two tubes), on an agar slope.
„ growth in broth, from kidney, on an agar slope.
„ growth in broth, from liver, on an agar slope.

August 24, 1904. Typical growth obtained from colony of spleen, planted out in litmus milk and glucose. Litmus milk rendered alkaline, glucose not fermented.

August 26, 1904. Typical growth, agglutinating at once with dilute serum, obtained from heart's blood.

August 28, 1904. Typical growth, agglutinating at once with monkey serum, obtained from kidney.

The following chart represents the temperature curve :—



Monkey No. 66.

Note.—This experiment is probably an instance of direct absorption of *M. melitensis* through a crack or abrasion of the mucous membrane of the mouth. The period of incubation and the wave of fever correspond exactly with those of Monkey No. 72, which was infected with

M. melitensis through a crack in the mucous membrane over the incisor teeth.

Experiment VI.—Monkey No. 72.

To Differentiate between Absorption from the Mouth and Throat and Absorption from the Stomach and Intestines.

Monkey No. 72 arrived in the laboratory on September 10, 1904. The blood was tested and gave no reaction with the *M. melitensis*. Feeding was then commenced, infected milk being passed directly into the stomach by means of an indiarubber tube. The growth on one agar slope, second generation, spleen of H——, incubated for 6 days at 37° C., was employed.

September 13, 1904. The feeding was continued as before, the growth on one agar slope, incubated for 7 days, being given. A small quantity of the milk regurgitated into the mouth, but no abrasion could be seen on the mucous membrane.

September 14, 1904. The growth from one agar slope, incubated for 8 days, was given.

September 15, 1904. The feeding was continued as before.

September 16, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given. A little milk again regurgitated into the mouth, and, on examination, a small crack was found in the mucous membrane opposite the upper incisor teeth. The mouth was at once washed out with lysol. The blood was examined but no reaction with the *M. melitensis* was obtained.

September 17, 1904. The feeding was continued, the growth from one agar slope, incubated for 5 days, being given.

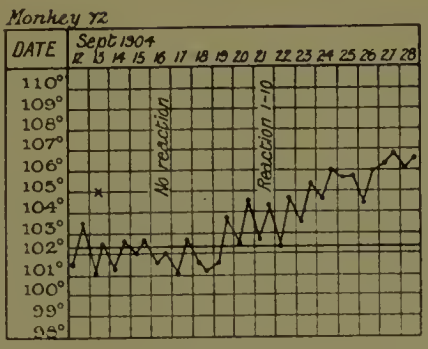
September 18, 1904. The growth from one agar slope, incubated for 6 days, was given.

September 21, 1904. The blood was examined and the serum in a dilution of 1—10, caused instantaneous clumping of the *M. melitensis*.

September 26, 1904. The serum, diluted 1—100, was found to agglutinate the *M. melitensis* instantaneously, the reaction being visible with the naked eye.

Note.—This monkey was directly infected either on September 13 or 16, the short incubation and sharp rise of temperature correspond to what is seen when the *M. melitensis* is directly absorbed into the peripheral circulation. Owing to the regurgitation of the infected milk into the mouth the experiment failed to differentiate between absorption from the mouth and from the alimentary canal; it, however, explains what probably occurred in the case of Monkey No. 66.

The prolonged incubation or rather slow absorption observed in the case of Monkeys Nos. 39, 40, and 41 forms a marked contrast to the rapid infection noticed in Monkeys Nos. 66 and 72, and approximates very closely to the results obtained when human beings are infected under natural conditions.



Monkey No. 72.

Experiment VII.—Monkey No. 45.

To note the Effect of the Subcutaneous Inoculation of M. melitensis *in Healthy Monkeys, and to Obtain a Specific Serum.*

July 9, 1904. Injected ½ c.c. of emulsion from an agar tube, second generation, from spleen of man. The agar tube was incubated for 6 days at 37° C., and the whole of the growth was used for the emulsion.

July 15, 1904. Complete agglutination with *M. melitensis* serum, diluted 1—10, and up to 1—160. No reaction with a dilution of 1—300.

July 21, 1904. Monkey looks ill. Tested serum—complete reaction, naked eye at once, dilution 1—1000. Shaved hair on back, and Zammit applied two female *Stegomyia*, which fed voraciously.

July 22, 1904. Zammit's feeding experiments with mosquitoes continued.

July 23, 1904. Zammit's feeding experiments with mosquitoes continued.

July 26, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

August 1, 1904. Tested serum; complete agglutination to naked eye, within 1 minute, dilution 1—1000.

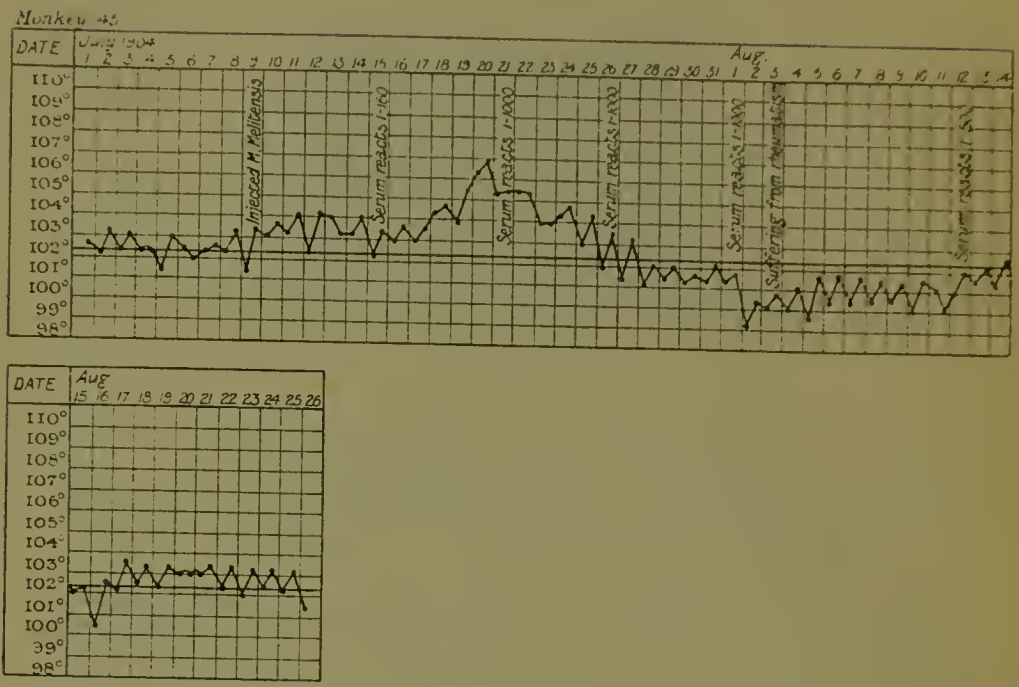
August 3, 1904. Monkey suffering from rheumatism (?); right arm and right wrist joint painful.

August 12, 1904. Examined blood; serum reacted at once, visible to naked eye, dilution 1—100; reaction after 5 minutes, dilution 1—500; dilution 1—1000, no reaction 5 minutes; feeble reaction, under microscope, after ½ hour.

September 9, 1904. Killed the monkey with chloroform. *Post-mortem*: Spleen much enlarged. Liver and kidneys congested. Other viscera healthy. Made cultures from the spleen, kidney and liver.

September 13, 1904. Recovered *M. melitensis* from spleen.

The following chart represents the temperate curve :—



Monkey No. 45.

Result.—The monkey suffered from a typical attack of Mediterranean fever.

Experiment VIII.—Monkey No. 48.

To Note the Effect of the Injection of Washings of Dust derived from Sergeants' Mess, Melleha Camp.

July 16, 1904. Dried soil (dust) from ventilation aperture, between w.c. and dining room of sergeants' mess at Melleha, received from Dr. Johnstone.

Soil macerated in sterile water, filtered, soil remaining washed, filtrate treated as follows :—10 c.c. injected into Monkey No. 48, subcutaneously between shoulders.

July 18, 1904. Ten cubic centimetres of further washings injected.

July 23, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 11, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

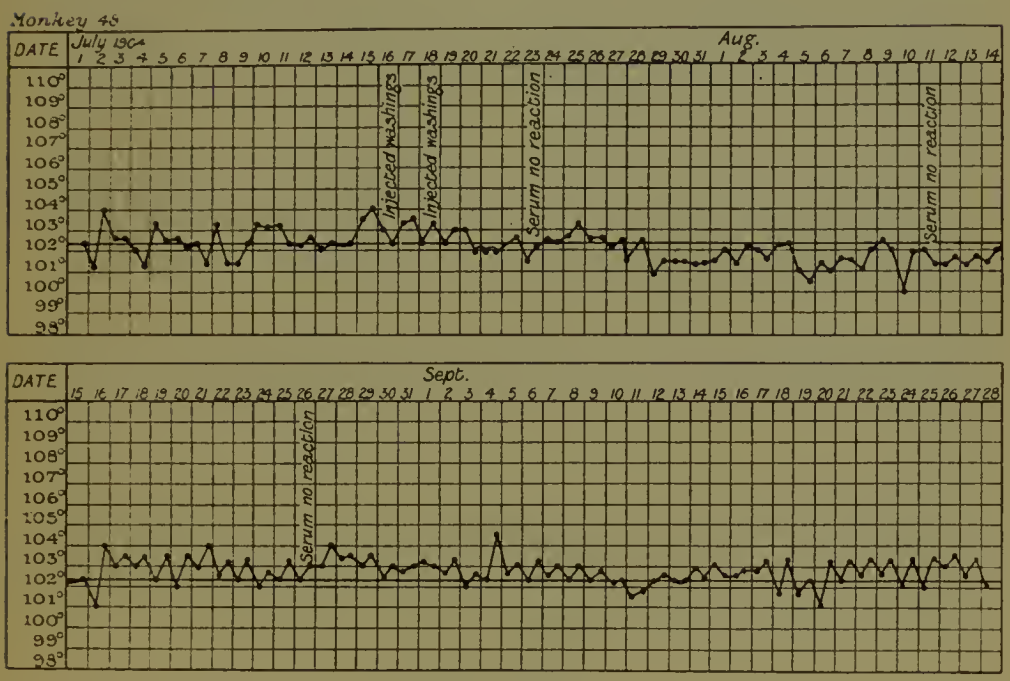
August 26, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

This experiment was performed at the request of Dr. Johnstone. The sergeants' mess at Melleha appeared to be the probable centre of infection of the sergeants of the Essex regiment. A disused w.c. was

found communicating by a ventilating aperture with the mess room. The dust was derived from this ventilating aperture.

The following chart represents the temperature curve :—



Monkey No. 48.

Result.—The *M. melitensis* was not present in the dust removed from the ventilating aperture.

Experiment IX.—Monkey No. 43.

To Note the Effect of the Injection of Washings of Supposed Infected Soil into a Healthy Monkey.

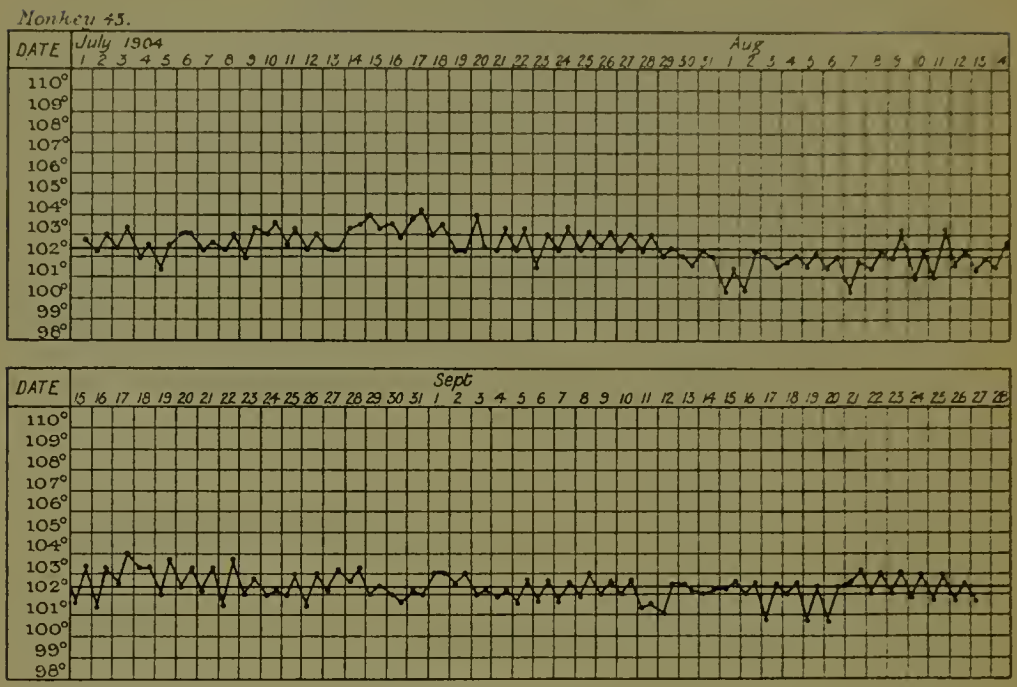
July 16, 1904. Dr. Johnstone forwarded 0·14 gramme of soil, obtained from the pan of the disused w.c. in the sergeants' mess, Melleha Camp. The soil was macerated in sterile water, filtered through paper, and the deposit again thoroughly washed. The total filtrate obtained was 20 c.c. Of this 10 c.c. was injected subcutaneously into a monkey.

July 18, 1904. The remainder of the washings injected.

July 23, 1904. Examined blood; serum, diluted 1—10, gave no reaction with *M. melitensis*.

August	1,	,,	,,	,,	,,
,,	10,	,,	,,	,,	,,
,,	17,	,,	,,	,,	,,
,,	26,	,,	,,	,,	,,
September	6,	,,	,,	,,	,,

The following chart represents the temperature curve :—



Monkey No. 43.

Result.—The *M. melitensis* was not present in the soil removed from the w.c. in the sergeants' mess.

Experiment X. Monkey No. 46.

Injection of Washings from Wall of an Infected House.

July 7, 1904. The walls of the w.c., No. 26 Strada Nuova, Sliema, where two cases of Mediterranean Fever occurred, were rubbed with cotton wool moistened with saline solution; the water was expressed and filtered through paper. Filtrate, collected in a sterile tube, was treated as follows :—

Injected 10 c.c. of filtrate.

July 8, 1904. Injected 10 c.c. of filtrate.

July 9, 1904. ,, ,,

July 10, 1904. Injected the remaining portion (8 c.c.) of filtrate.

July 16, 1904. Examined blood, no reaction with *M. melitensis*, dilution 1—10.

July 18, 1904. Washings from kitchen, grown in broth for 11 days, injected to-day.

July 26, 1904. Tested serum, no reaction with *M. melitensis*, dilution 1—10.

August 10, 1904. Examined serum, no reaction with *M. melitensis*, dilution 1—10.

September 6, 1904. Examined blood; serum reacts at once with *M. melitensis*, dilution 1—10; reaction 1—500, after waiting 15 minutes.

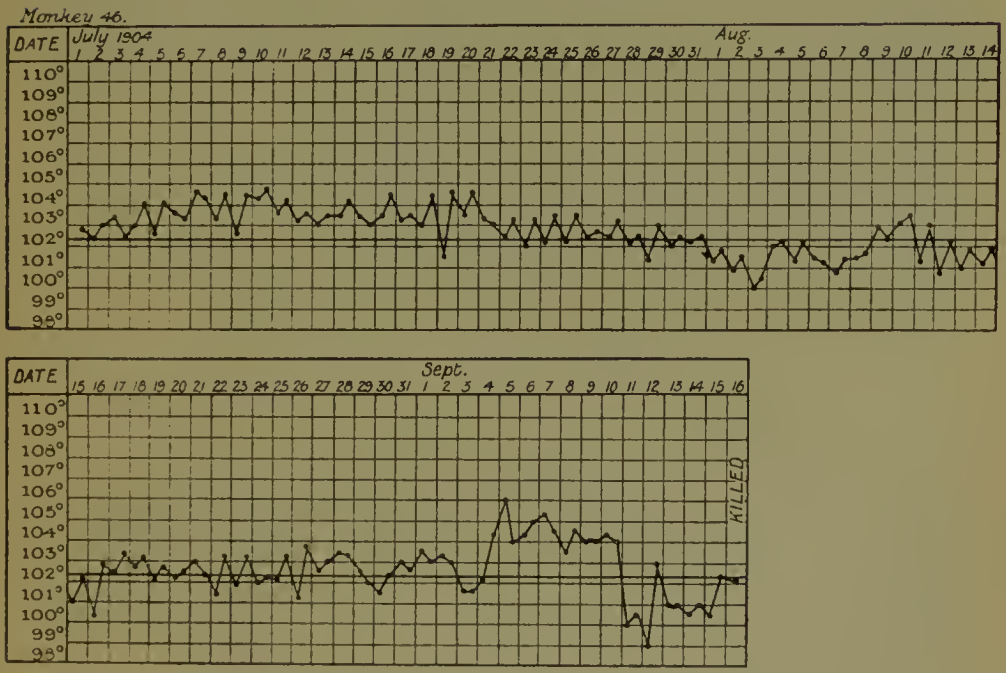
September 15, 1904. Examined blood; serum reacts at once in a dilution of 1—500; in a dilution of 1—1000 a reaction, visible to the naked eye, is seen in 5 minutes.

September 16, 1904. Killed the monkey with chloroform.

Post-mortem.—Spleen enlarged, but firm in consistence; kidneys and liver congested; pericardium contained a little fluid; other viscera healthy. Cultures made from spleen, liver, kidney, heart's blood, and urine.

September 23, 1904. *M. melitensis* isolated from spleen, kidney, and urine.

The following chart shows the temperature curve:—



Monkey No. 46.

Remarks.—This result is probably due to infection conveyed from neighbouring monkeys. Even if the *M. melitensis* had been present in the growth injected on July 18, it is highly improbable that the specific microbe when injected subcutaneously would have remained latent for a period of 50 days. Monkey No. 69 has also become infected since its arrival, without receiving the specific microbe either by the mouth or subcutaneously.

Monkey No. 46 on one side is next to Monkey No. 45, which received *M. melitensis* subcutaneously and developed a typical attack of fever.

On the other side of Monkey No. 46 is Monkey No. 47, infected by dust blown into the throat. Evidently this monkey has become infected, either by personal contact, by urine, or by means of *Stegomyia*.

Experiment XI.—Monkey No. 42.

To Determine if the Subcutaneous Injection of Infected Urine from a Case of Mediterranean Fever will give rise to the Disease in a Monkey.

July 13, 1904. Injected 10 c.c. of Howe's urine, enriched with broth, and incubated for 14 days at 37° C. (3 c.c. urine).

July 14, 1904. Injected 10 c.c. of Howe's urine (3 c.c. urine) treated as above, but incubated 15 days.

July 15, 1904. Injected 10 c.c. of mixed urine and broth (3 c.c. of urine), incubated 14 days.

July 18, 1904. Examined blood. Feeble reaction with one culture, blood diluted 1—10; tested with another culture, no reaction was obtained.

July 19, 1904. Injected 5 c.c. of broth culture, made at *post-mortem* of Howe by adding 1 c.c. of urine from bladder to broth, and then incubating at 37° C. for 12 days. Examined by hanging drop; fine cocci and chains, corresponding in morphology to *M. melitensis*, observed, the cocci decolorised by Gram.

July 20, 1904. Injected 10 c.c. of broth culture, made at *post-mortem* by adding contents of right ureter to a broth tube.

July 25, 1904. Examined blood; no reaction with *M. melitensis*, dilution 1—10.

August 2, ,, ,, ,, ,,

,, 11, ,, ,, ,, ,,

,, 26, ,, Examined blood; reacts 1—10 at once.

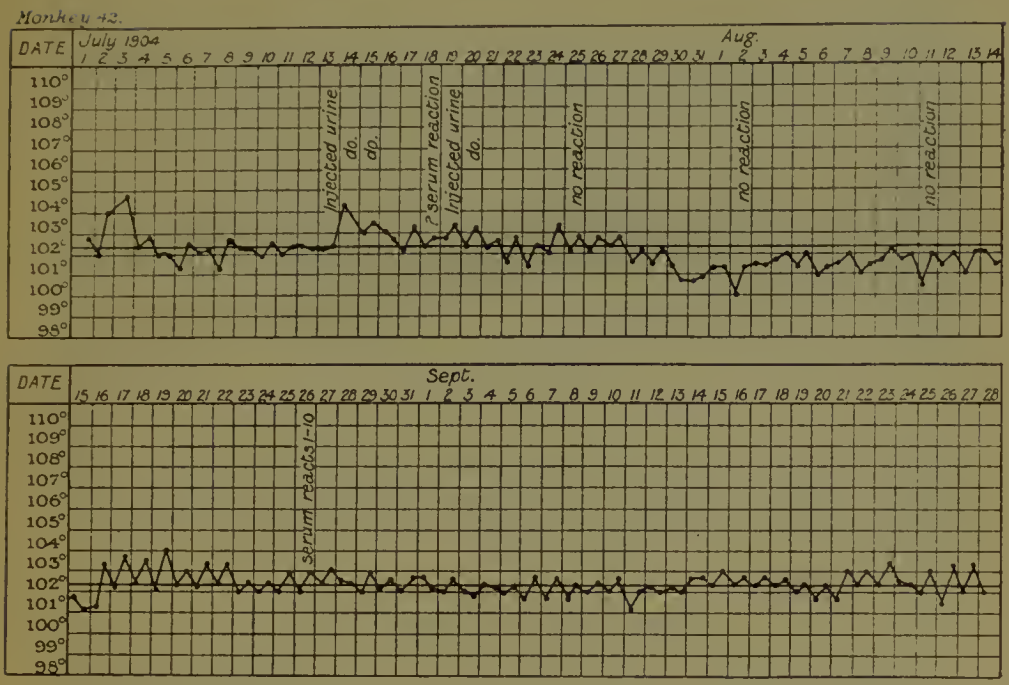
September 7, 1904. Examined blood; serum reacts in dilution of 1—20 at once: dilution 1—100, no reaction.

September 27, 1904. Killed monkey; made cultures from spleen, liver, kidney, heart's blood, and urine.

October 10, 1904. All the cultures have remained sterile.

Note.—The *M. melitensis* was recovered by plating another sample of the urine, removed from the bladder at the *post-mortem*.

The following chart shows the temperature curve; it will be noticed that there has never been a wave of fever, the slight serum reaction was probably caused by toxines contained in the urine:—



Monkey No. 42.

Remarks.—The *M. melitensis* was probably not present in the specimens of urine injected into this monkey. The slight blood reaction obtained might be caused by toxines in the urine.

Experiment XII.—Monkey No. 55.

To Determine whether Cultures of M. melitensis, *Derived from Infected Urine, will give Rise to the Disease in a Monkey.*

July 29, 1904. Growth from Pudney's urine, third generation, grown for 3 days on agar slope (glucose-litmus-nutrose-agar). The whole of the growth diffused in 2 c.c. of broth, and injected into this monkey.

August 4, 1904. Examined blood; complete instantaneous reaction, visible to naked eye, blood dilution 1—10; no reaction 1—50.

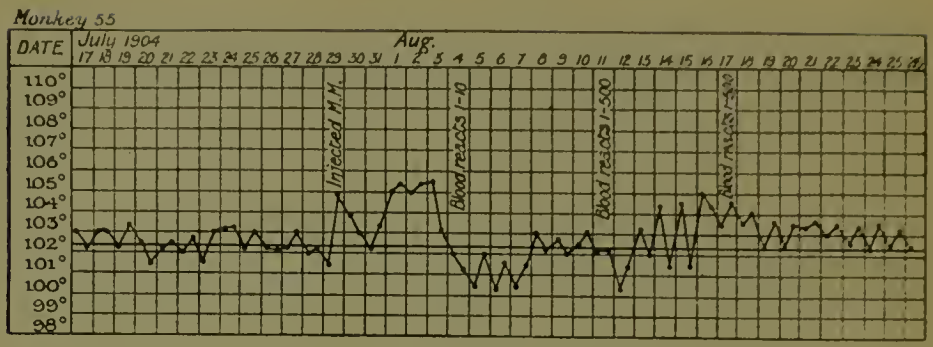
August 11, 1904. Examined blood; complete instantaneous reaction visible to naked eye, dilution 1—100; after 5 minutes, reaction visible in dilution 1—500.

August 17, 1904. Examined blood; reaction as on August 11, 1904.

September 8, 1904. Killed the monkey to-day. *Post-mortem*: Spleen enlarged and friable. Kidneys congested. Other viscera apparently healthy. Made cultures from the spleen, liver and kidneys.

September 12, 1904. *M. melitensis* recovered from the spleen.

The following chart shows the temperature curve:—



Monkey No. 55.

Result.—This experiment shows that the *M. melitensis* recovered from the urine of Mediterranean Fever patients is capable of giving rise to the disease in healthy monkeys.

Experiment XIII.—Monkey No. 69.

Is Mediterranean Fever Conveyed from Diseased to Healthy Monkeys by Contact?

This monkey arrived in the laboratory on August 7, 1904. It was placed in a cage, between Monkey No. 41, infected by dust, and Monkey No. 40, infected by feeding. Monkey No. 69 appeared perfectly healthy on arrival, and ate well; its temperature was taken from August 9, and after August 16 displayed an erratic course, probably accounted for by the intense heat of the terrace from early morning until evening.

On August 26, 1904, the blood was examined, but the serum, diluted 1—10, gave no signs of reaction with *M. melitensis.*

On September 7, 1904, the blood was again examined, and the serum, diluted 1—10, caused immediate clumping of the *M. melitensis*, visible to the naked eye.

Since September 9, 1904, the monkey has been obviously ill, losing flesh and sitting "moping" in his box all day.

On September 11, 1904, the serum, diluted 1—20, caused instantaneous clumping of the *M. melitensis.*

On September 13, 1904, the monkey died, much emaciated.

Post-mortem examination: All the viscera appeared healthy; cultures were made from the spleen, liver, kidneys, heart's blood and urine. *M. melitensis* isolated from the spleen and liver.

Remarks as to the Mode of Infection of this Monkey. — It seems possible that it might have occurred in three ways, *i.e.* (*a*) by direct personal contact; (*b*) by direct infection from walking in the infected urine of his neighbours; (*c*) by means of *Stegomyia.* When at full

length of his chain, Monkey No. 69 could touch either of his neighbours and walk on the ground infected by them.

If personal contact alone had been the cause of infection, Monkey No. 48 ought to have been infected by Monkey No. 47. Also the *M. melitensis* has not yet been isolated from the sweat or skin scrapings of patients suffering from Mediterranean fever.

If the infection had been carried by *Stegomyia*, there should have been a general infection amongst the monkeys on the terrace. There appears no reason why mosquitoes should have picked out Monkey No. 69 and Monkey No. 46, which also appears to have been infected by its neighbours. At this time there were six other healthy monkeys on the terrace exposed to the bites of mosquitoes, and one of them, No. 48, was in a cage next to an infected monkey. Yet none of these monkeys have shown the slightest trace of a blood reaction. Direct infection through infected urine seems to be the most probable explanation of the infection. Both Monkey No. 69 and Monkey No. 46 had infected monkeys next to them, and the chance of infection from urine was undoubted, as the *M. melitensis* was discovered in the urine of Monkey No. 46, proving that the specific microbe is excreted from monkeys in the same manner as from human beings. Although the cages and cemented surfaces beneath them were washed with lysol night and morning, still the ground was often noticed covered with decomposing urine.

Having in view the possibility of direct infection from urine excreted by monkeys suffering from Mediterranean fever, it is necessary to enquire whether any of the experiments previously recorded are invalidated by this circumstance. It will be advisable to discuss the experiments *seriatim*.

Experiment I, Monkey No. 41.—This monkey was kept in a small room on the left of the door leading from the laboratory to the roof. It was not placed in its box until infection had been acquired, and even after this it was still separated from Monkey No. 40 by a healthy monkey. It is evident that in relation to this experiment the question of infection by urine could not arise.

Experiment II, Monkey No. 47.—This monkey was placed between two healthy monkeys, viz., No. 46 and No. 48. Monkey No. 48 remained in good health throughout the summer and never showed the slightest sign of infection. Monkey No. 47 was infected on August 8, 1904, but Monkey No. 46 did not show a reaction until September 6, 1904. It is obvious that Monkey No. 47 could not have been infected by urine excreted by its neighbours.

Experiment III, Monkey No. 39.—The monkey was placed between Monkey No. 58 and Monkey No. 66. Monkey No. 58 only received injections of filtered toxines, and could not possibly excrete the specific micrococci in its urine. Monkey No. 66 was directly infected through

a crack in the mouth, and suffered from a marked bacterial infection; its first rise of temperature occurred on August 10, 1904, and it is practically impossible that the *M. melitensis* could have been excreted in its urine before this date, and, taking into consideration the facts observed in man, it is unlikely that the urine would contain the *M. melitensis* before August 25, 1904. Consequently it seems impossible that the Monkey No. 39 could have received infection from the urine of its neighbours.

Experiment IV, Monkey No. 40.—This monkey was infected on August 11, 1904, and the monkeys nearest to it, viz., 69 and 41, were not infected until September 7, 1904, August 26, 1904, respectively. The question of infection by urine could not arise in this case.

Experiment V, Monkey No. 66.—This monkey was placed between Monkey No. 67 and Monkey No. 39. Monkey No. 67 never showed the slightest trace of infection, and was in good health all the summer. Monkey No. 39, as previously stated, was infected about the same date as Monkey No. 66. It does not seem possible that infection by urine could have played a part in this experiment.

Experiment VI, Monkey No. 72.—This monkey was directly infected through a crack in the mucous membrane of the mouth on September 13 or 16. It was kept apart from infected monkeys.

Experiment VII, Monkey No. 45.—This monkey was directly infected by subcutaneous injection of the *M. melitensis*.

Experiment VIII, Monkey No. 48 } These monkeys failed to become
Experiment IX, Monkey No. 43 } infected.

Experiment X, Monkey No. 46.—This monkey was infected on September 6, 1904, and it appears practically certain that the infection was caused by the specific micrococci present in the urine of neighbouring monkeys.

Experiment XI, Monkey No. 42.—This monkey probably only received toxines contained in the urine excreted by a case of Mediterranean fever.

Experiment XII, Monkey No. 55.—This monkey was directly infected by the subcutaneous injection of the *M. melitensis*.

Experiment XIII, Monkey No. 69.—This monkey became infected on September 7, 1904. The source of infection was probably the urine of its neighbours.

List of Monkeys, not infected, artificially infected, and naturally infected, with Dates of Arrival and Infection.

No.	Infection.	Arrival.	Remarks.
70.	Not infected.	8/8/04.	
65.	,,	,,	Dr. Zammit's mosquito experiments.
64.	,,	,,	,, ,,
63.	Artificially infected.	,,	,, ,,
62.	Not infected.	16/7/04.	
61.	,,	,,	
60.	,,	,,	Died, diarrhœa, 26/8/04.
59.	,,	,,	Died 11/9/04.
49.	,,	8/8/04.	
48.	,,	1/7/04.	
47.	Artificially infected 9/8/04.	,,	Died. Experiment II, page 48.
46.	Naturally infected 6/9/04.	,,	
45.	Artificially infected 15/7/04.	,,	Subcutaneous injection 9/7/04.
44.	Not infected.	,,	Died from pneumonia, 6/7/04.
43.	,,	,,	
42.	(?) Infected (probably toxine)	,,	Urine infection.
67.	Not infected.	8/8/04.	Mosquito experiment.
66.	Artificially infected ? 9 or 10/8/04.	,,	Food experiment. Serum 13/8/04.
39.	Artificially infected 10/8/04.	1/7/04.	Food experiment.
58.	Not infected.	16/7/04.	Toxine injected.
57.	,,	,,	Died from diarrhœa 15/8/04.
56.	,,	,,	,, 5/8/04.
55.	Infected 4/8/04.	,,	Culture from urine. Serum 4/8/04.
54.	Not infected.	,,	Skin scraping.
68.	,,	8/8/04.	,,
40.	Artificially infected 11/8/04.	1/7/04.	Experiment IV, page 53.
69.	Naturally infected 7/9/04.	8/8/04.	
41.	Artificially infected 26/8/04.	8/7/04.	Died. Experiment I, page 46.

Mosquito Experiments.

These experiments were undertaken in order to ascertain whether the *Stegomyia fasciata* is able to convey the *M. melitensis* from the peripheral blood of Malta Fever patients to healthy monkeys.

Experiment I.

In this experiment the mosquitoes were fed on Private Lawrence, 2nd Essex Regiment. This particular patient was selected, as Staff-Surgeon Shaw had found the maximum number of micrococci in his blood. The number of mosquitoes and the dates on which they were

fed on the patient and on Monkey No. 70, are shown in Table I. An endeavour was made to keep the mosquitoes alive as long as possible, as in view of the work done on Yellow Fever it seemed possible that several days might intervene between the absorption of the *M. melitensis* into the stomach of the mosquito and its transfer, possibly through the salivary glands, to the proboscis. In Dr. Zammit's successful experiment only 48 hours intervened between the absorption of the micrococci and their transfer to the patient. In Experiment I the intervals were 2, 4, 8, and 10 days, respectively. Monkey No. 70 had been under observation for several months and always appeared perfectly healthy. Its serum was examined at varying periods, but it never manifested the slightest power of agglutinating the *M. melitensis*.

Experiment II.

The same procedure was followed in this experiment, the patient, Private K——, R.A.M.C., having a typical wave of fever. The number of mosquitoes and the dates when they were fed on the patient and on Monkey No. 44, are given in Table II. The mosquitoes were kept alive for 13 days, and yet no trace of agglutination could be detected when the serum of the monkey, in a low dilution, was added to an emulsion of the *M. melitensis*.

Experiment III.

In this experiment mosquitoes were fed on different patients, specially selected owing to the presence of marked fever at the time of feeding. The details of the various feedings are given in Table III. All the agglutination tests were negative.

Experiment IV.

In this experiment mosquitoes were fed on monkeys recently inoculated with *M. melitensis* and, after an interval of 48 hours, transferred to Monkey No. 76 which arrived at the laboratory on 8.9.04. On 16.9.04 and 22.9.04 the serum of Monkey 76, diluted 1—10, was added to an emulsion of *M. melitensis*; no agglutination was observed on either occasion. On the 20.9.04 mosquitoes were fed on Monkey No. 60A, at that time at the summit of a wave of fever, and 48 hours later they were fed on Monkey No. 76. On the 25.9.04, mosquitoes were again fed on Monkey No. 60A, and on the 27.9.04 transferred to Monkey No. 76. On the 27.9.04 mosquitoes were fed on Monkey No. 72, infected by feeding and at the height of a wave of fever, and 48 hours later transferred to Monkey No. 76. The serum of Monkey No. 76 was examined on 27.9.04, but did not manifest the slightest power of agglutinating the *M. melitensis*.

(These experiments are still proceeding.)

Table I.—Monkey No. 70. Mosquito Experiments (continued up to the end of October).

Mosquitoes fed on patient.		Mosquitoes fed on monkey.																																								
Date.	No. of mos-quitoes.	No. of days after being fed on patient.																																								No. of times each mosquito fed on monkey.
		1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	23	24	25	26	27	28	29	30	31	32	33	34	35	36	37	38	39	40	
1904.																																										
Sept. 6..	2 { 1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
	1	..	1	..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
" 9..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
" 10..	1	..	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
" 17..	2 { 1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
	1	..	1	..	1	..	1	..	1	..	1	..	1	..	..	..	1	..	..	1	..	1	..	1	..	..	1	..	1	..	1	..	..	1	..	1	..	1	..	1	..	17

The monkey was bitten 24 times by presumably infected mosquitoes, and, in the case of one mosquito, 39 days intervened between the first feeding on the patient and the last feeding on the monkey. The serum was repeatedly examined, but never caused the slightest agglutination of the *M. melitensis.*

Table II.—Monkey No. 44. Mosquito Experiments (continued up to the end of October).

Mosquitoes fed on patient.		Mosquitoes fed on monkey. No. of days after being fed on patient.																				No. of times each mosquito fed on monkey.
Date.	No. of mosquitoes.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	
1904.																							
Aug. 29..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
.. 31..	2	1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
		1	..	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
Sept. 14..	4	1	..	1	..	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	3
		1	..	1	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	4
		1	.	1	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	4
		1	..	1	..	..	1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	6
„ 17..	2	1	..	1	..	..	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	4
		1	..	1	..	1	..	1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	6
Oct. 5..	2	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	1
		1	..	1	..	1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	5
„ 5..	4	1	..	1	..	..	1	..	1	..	1	..	..	1	..	1	..	1	..	1	..	..	8
		1	..	1	..	1	..	1	..	1	..	1	..	..	..	1	..	1	..	1	..	..	9
		1	..	1	..	1	..	1	..	1	..	1	..	..	..	1	..	1	..	1	..	1	10
		1	..	1	..	1	..	1	..	1	..	1	..	..	..	1	..	1	..	1	..	1	10
„ 5..	2	1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
		1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	4
„ 24..	4	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	..	2
		1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
		1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3
		1	..	1	..	1	..	1	..	..	..	..	..	..	..	..	..	..	..	..	..	..	3

This monkey was bitten 92 times by presumably infected mosquitoes, and in the case of two mosquitoes 21 days intervened between the first feeding on the patient and the last feeding on the monkey. Its serum was repeatedly examined, but never caused the slightest agglutination of the *M. melitensis*.

Table III.—Monkey No. 56. Mosquito Experiments (continued up to the end of October).

Mosquitoes fed on patients.		Mosquitoes fed on monkey. Number of days after being fed on patient.																						Number of times each mosquito fed on monkey.
Date.	No. of mosquitoes.	1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	20	21	22	
1904.																								
Sept. 4	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	..	...	...	...	...	1
,, 16	3	...	...	1	...	...	...	...	...	..	...	...	..	...	...	...	...	...	...	...	...	...	...	1
		...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
		...	...	1	...	...	...	...	...	...	..	...	...	...	...	...	...	...	...	...	...	...	...	1
,, 22	1	...	..	1	.	1	...	...	1	...	...	1	...	...	1	...	1	...	1	...	1	...	1	9
,, 23	4	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
		...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	1	...	1	...	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	4
		...	1	...	1	...	...	1	...	...	1	...	...	1	...	1	...	...	...	...	...	...	...	6
,, 25	3	...	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
,, 26	4	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
		...	...	1	...	...	...	...	.	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
		...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	1
		...	...	1	...	...	...	1	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	4
Oct. 10	6	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
		...	1	...	1	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4
		...	1	...	1	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	4
		...	1	..	1	...	1	...	1	...	1	...	1	...	...	...	...	...	...	...	...	...	...	6
		...	1	...	1	...	1	...	1	...	1	...	1	...	1	...	1	...	...	...	...	...	...	8
		...	1	...	1	...	1	...	1	...	1	...	1	...	1	...	1	...	1	...	...	1	...	10
,, 19	4	...	...	1	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	2
		...	...	1	...	1	...	...	...	...	...	...	...	...	...	.	...	...	...	...	...	...	...	2
		...	...	1	...	1	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	5
		...	...	1	..	1	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	5
,, 24	4	...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	1	.	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3
		...	1	...	1	...	...	1	...	...	...	...	...	...	...	...	...	...	...	...	...	...	...	3

Monkey No. 56 was bitten 101 times by presumably infected mosquitoes Its serum was repeatedly tested as to agglutination of the *M. melitensis*, but no signs of a reaction were observed.

The want of success, which has up to the present attended our efforts to transfer by means of mosquitoes the *M. melitensis* from infected human beings to healthy monkeys, is disappointing but does not necessarily invalidate the result obtained by Dr. Zammit. The case upon which he made his successful experiment was unusually severe, and since then cases of this type have not been met with either in the military or in the civil hospitals.

Conclusions drawn as to the Mode of Entrance of the M. melitensis *into the Body.*

There is experimental evidence to show that the *M. melitensis* when present in dry dust is capable of being absorbed by monkeys.

The path of absorption may be through the nares, throat, respiratory passages, and alimentary canal. When present in food it is also taken into the system of monkeys; here, again, the path of absorption may be through the throat as well as through the mucous membrane of the alimentary canal.

When transmitted through an unbroken mucous membrane the process of absorption is comparatively slow, and under these conditions the wave of fever appears to be prolonged. The long and variable incubation period observed in monkeys infected through an unbroken mucous membrane is frequently observed in man infected under natural conditions.

When the *M. melitensis* is absorbed through a crack in a mucous membrane or in the skin, or is injected subcutaneously, the absorption is rapid and the incubation period in monkeys varies from 5 to 7 days. The curve of fever is characterised by a rapid rise usually followed by a rapid fall. These acute infections have also been observed in man infected under the same conditions, but the period of incubation appears to be longer in man than in the monkey.

The history of Monkeys Nos. 69 and 47 shows that healthy monkeys may become infected by urine secreted by monkeys suffering from Mediterranean Fever. Just as in the case of man, the *M. melitensis* is excreted in the urine of infected monkeys. And it seems probable that healthy monkeys walking in the infected secretion convey the specific microbe into the mouth by means of the paws.

Infection by means of urine secreted by cases of Mediterranean Fever readily explains the cases of Mediterranean Fever which appear to arise spontaneously in hospitals. In the absence of specific knowledge as to the mode of excretion of the *M. melitensis* from the human body, sufficient care has hitherto not been taken to sterilise bed-pans, urine bottles and sheets soiled by cases of Mediterranean Fever.

There is no evidence that Mediterranean Fever can be contracted by contact with cutaneous surfaces, uncontaminated by urine.

The experiments made with *Stegomyia fasciata* do not support the result obtained by Dr. Zammit.

5.

DESCRIPTION OF A METHOD OF CULTIVATING THE *MICROCOCCUS MELITENSIS* FROM SMALL QUANTITIES OF PERIPHERAL BLOOD AND INOCULATION EXPERIMENTS WITH THE MICRO-ORGANISMS ISOLATED.

By Staff-Surgeon R. T. Gilmour, R.N., Bighi Hospital, Malta.

[*Note.*—This work was kindly undertaken by Staff-Surgeon Gilmour, R.N., at the laboratory of the Naval Hospital, Malta. He has already published a paper on the subject entitled "A few Notes on the Bacteriology and Pathology of Mediterranean Fever," published in 'Health of the Navy' for 1902. In that paper he gives the result of the examination of sixteen cases of Mediterranean Fever. Out of these sixteen cases the *M. melitensis* was isolated from eight, three gave no growth, and five were uncertain as they were contaminated. In these first experiments Staff-Surgeon Gilmour used fairly large quantities of blood and incubated the blood in a large volume of broth. From 0·5—8·8 c.c. blood in from 15—60 c.c. of broth were used.—Ed.]

Preparation of the Patient.

The arm should be chosen in which the veins at the bend of the elbow are the most prominent. The selected limb should be shaved from the middle of the arm to the middle of the forearm. This area should then be washed with hot sterile water, carbolic soap, and a sterile nail-brush for 20 minutes; then swabbed with ether for 10 minutes, to dissolve out the fat, and finally scrubbed with a 1 in 500 solution of perchloride of mercury for ¼ hour. A sterile dressing should then be applied, soaked in the same disinfectant, until the time of the operation, about 24 hours afterwards.

The Apparatus Required.

1. A sterile bandage.
2. A sterile 10 c.c. serum syringe.

3. (*a*) One flask, containing 30 c.c. of broth.
   (*b*) Two tubes, each containing 9 c.c. of broth.*
   (*c*) Sufficient Petri's dishes, each containing 10 c.c. of agar-agar.
4. A spirit lamp.
5. Sterile 1 c.c. pipettes and glass rods.
6. Six tubes, each containing 10 c.c. of broth.

Method of Extracting the Blood.

1. Remove the bandage from the dressing.
2. Constrict the arm above the elbow-joint with the sterile bandage.
3. After waiting a few minutes, so that the veins may become engorged, insert the needle into the most prominent vein and withdraw sufficient blood, about 5 c.c.
4. An assistant, holding the flask and the tubes on the slant, should then remove the plugs with sterile forceps, and the required quantities of blood (2 c.c. for the flask, and 1 c.c. for the two 9 c.c. tubes) should be passed into the broth.

The assistant should then keep the broth in the tubes well agitated, so as to prevent coagulation and get a good emulsion.

0·5 c.c. of blood should then be passed into each of the Petri dishes, and immediately spread out with a sterile rod.†

5. The next part of the procedure must be performed in the laboratory. Pass the following quantities of emulsion, from one of the 9 c.c. tubes, into others containing 10 c.c. of broth :—‡

0·1	c.c. of	emulsion	=	(0·01	c.c. of	blood)	into the	1st tube.
0·25	,,	,,	=	(0·025	,,	,,)	,,	2nd ,,
0·5	,,	,,	=	(0·05	,,	,,)	,,	3rd ,,
1·0	,,	,,	=	(0·1	,,	,,)	,,	4th ,,
2·0	,,	,,	=	(0·2	,,	,,)	,,	5th ,,
3·0	,,	,,	=	(0·3	,,	,,)	,,	6th ,,

6. Incubate the broth tubes and Petri's dishes at 35° C., and examine daily. From the 4th to 10th day of incubation inoculate sloped agar tubes from the broths, allowing 15 drops to flow over the surface of each. Ring all colonies daily, which appear in the Petri dishes, and number them, keeping a tally of the day they appeared. From the 4th to the 10th day remove the colonies with a sterile loop, plant on agar, and incubate at 35° C.

The following are the tests applied to ascertain whether a growth is *M. melitensis* :—

* Tubes containing 19 c.c. of broth were afterwards used.

† These dishes were afterwards inoculated with 1 c.c. of 1—10 emulsion.

‡ Smaller quantities of blood were afterwards used.

1. An emulsion in normal saline is examined under the microscope.
2. Specimens are stained with Neelson's carbol-fuchsine (1 in 10).
3. Specimens are stained by Gram's method.
4. The growth is tested for agglutination with the sera of Mediterranean fever cases; controls being made with healthy serum.

The reaction of all media used in the experiments is +10A (Eyre's scale) unless otherwise stated.

Experiment I.

Harry Chapman, 28. Admitted into hospital on April 2, 1904. On June 23, 1904, the 84th day of illness, 8·0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
4·0 c.c.	30 c.c.	Pure culture of *M. melitensis.*
1·0 ,,	10 ,,	,, ,,
1·0 ,,	9 ,,	Used for inoculating the following tubes.
0·01 ,,	10 ,,	Negative.
0·025 ,,	10 ,,	,,
0·5 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·2 ,,	10 ,,	,,
0·3 ,,	10 ,,	,,

Result of Inoculations of Blood on to Sloped Agar Tubes.

Amount of blood used.	Amount of medium used.	Result.
0·5 c.c.	10 c.c.	One colony of *M. melitensis.*
,,	,,	Three colonies of *M. melitensis.*
,,	,,	Sterile.

Experiment II.

J. S. Ward, 24. Admitted into hospital on June 8, 1904. On June 25, 1904, the 17th day of illness, 5·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2·0 c.c.	30 c.c.	Contaminated.
1·0 ,,	9 ,,	The *M. melitensis* obtained.
0·01 ,,	10 ,,	Sterile.
0·025 ,,	10 ,,	,,
0·05 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·2 ,,	10 ,,	The *M. melitensis* obtained.
0·3 ,,	10 ,,	Sterile.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·5 c.c.	10 c.c.	Five colonies of *M. melitensis* obtained.
0·25 ,,	10 ,,	Contaminated.

Experiment III.

Alfred Law, 20. Admitted into hospital on June 20, 1904. On June 28, 1904, the 14th day of illness, 5·0 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2·0 c.c.	30 c.c.	Contaminated.
0·01 ,,	10 ,,	Sterile.
0·025 ,,	10 ,,	*M. melitensis* obtained.
0·5 ,,	10 ,,	,, ,,
0·1 ,,	10 ,,	,, ,,
0·2 ,,	10 ,,	,, ,,
0·3 ,,	10 ,,	,, ,,
0·01 ,,	10 ,,	,, ,,
0·025 ,,	10 ,,	,, ,,
0·05 ,,	10 ,,	,, ,,
0·1 ,,	10 ,,	,, ,,
0·2 ,,	10 ,,	Broth contaminated.
0·3 ,,	10 ,,	,, ,,

Experiment IV.

John Waters, 23. Admitted into hospital on June 29, 1904. On July 5, 1904, the 25th day of illness, 8·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
2·0 c.c.	30 c.c.	The *M. melitensis* obtained.
1·0 ,,	9 ,,	Sterile.
0·01 ,,	10 ,,	,,
0·025 ,,	10 ,,	,,
0·05 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·2 ,,	10 ,,	,,
0·3 ,,	10 ,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·5 c.c.	10 c.c.	One colony of *M. melitensis*.
,,	,,	,, ,,
,,	,,	Contaminated.
,,	,,	Two small contaminations. No *M. melitensis*.
,,	,,	One small contamination. No *M. melitensis*.

Experiment V.

Thomas Eccles, 23. Admitted into hospital on July 9, 1904. On July 19, 1904, the 23rd day of illness, 2·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·01 c.c.	10 c.c.	Contaminated.
0·025 ,,	,,	,,
0·05 ,,	,,	,,
0·2 ,,	,,	,,
0·2 ,,	,,	,,

Result of Inoculations of Blood on to Sloped Agar Tubes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Contaminated.
,,	,,	,,
,,	,,	,,
,,	,,	,,
,,	,,	,,

The whole of these growths were contaminated with a staphylococcus.

Experiment VI.

Edward Stedman, 32. Admitted into hospital on July 7, 1904. On July 20, 1904, the 25th day of illness, 3·5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	One colony of *M. melitensis*.
,,	,,	Sterile.
,,	,,	Contaminated.
,,	,,	Sterile.

Experiment VII.

Sidney Fleetwood, 23. Admitted into hospital on June 11, 1904. On July 21, 1904, the 40th day of illness, 4·0 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1·0 c.c.	50 c.c.	Pure culture of *M. melitensis*.
0·01 ,,	10 ,,	Sterile.
0·025 ,,	10 ,,	,,
0·05 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·2 ,,	10 ,,	,,
0·3 ,,	10 ,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
,,	,,	,,
,,	,,	,,
,,	,,	,,
,,	,,	One colony of *M. melitensis*.

Experiment VIII.

James Slater, 21. Admitted into hospital on July 13, 1904. On July 22, 1904, the 20th day of illness, 3·5 c.c. of blood were withdrawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1·5 c.c.	30 c.c.	Pure culture of *M. melitensis*.
0·005 ,,	10 ,,	Sterile.
0·0125 ,,	10 ,,	,,
0·025 ,,	10 ,,	,,
0·05 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·15 ,,	10 ,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
,,	,,	Contaminated.
,,	,,	Sterile.
,,	,,	Contaminated.
,,	,,	,,
,,	,,	Sterile.

Experiment IX.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 12, 1904, the 3rd day of illness, 3·5 c.c. of blood were withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
1·0 c.c.	30 c.c.	Pure culture of *M. melitensis* obtained.
0·005 ,,	10 ,,	Sterile.
0·0125 ,,	10 ,,	,,
0·025 ,,	10 ,,	,,
0·05 ,,	10 ,,	,,
0·1 ,,	10 ,,	,,
0·15 ,,	10 ,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	One colony of *M. melitensis*, and one small colony of contamination.
,,	,,	One small colony of contamination.
,,	,,	Sterile.
,,	,,	Contaminated.
,,	,,	,,
,,	,,	,,

Experiment X.

Arthur Witte, 27. Admitted into hospital on August 9, 1904. On August 19, 1904, the 10th day of illness, 2·5 c.c. of blood were with drawn from the right median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·005 c.c.	10 c.c.	Sterile.
0·0125 ,,	,,	Contaminated.
0·025 ,,	,,	,,
0·05 ,,	,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
	,,	One small colony of contamination.
,,	,,	Contaminated.

Experiment XI.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 19, 1904, the 5th day of illness, 1·0 c.c. of blood was withdrawn from the left median-basilic vein.

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	31 colonies of *M. melitensis*.
,,	,,	33 ,, ,,
,,	,,	31 ,, ,,

Experiment XII.

Edward Freak, 21. Admitted into hospital on August 18, 1904. On August 22, 1904, the 24th day of illness, 1·0 c.c. of blood was withdrawn from the left median-basilic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·005 c.c.	10 c.c.	Sterile.
0·0125 ,,	,,	,,
0·025 ,,	,,	Contaminated.
0·05 ,,	,,	Sterile.
0·1 ,,	,,	,,

Experiment XIII.

Frank Murch, 26. Admitted into hospital on August 14, 1904. On August 27, 1904, the 13th day of illness, 3·0 c.c. of blood were withdrawn from the right median-cephalic vein.

Result of Inoculations of Blood into Broth Tubes.

Amount of blood used.	Amount of medium broth used.	Result.
0·0025 c.c.	10 c.c.	Sterile.
0·005 ,,	,,	,,
0·0125 ,,	,,	,,
0·025 ,,	,,	,,
0·05 ,,	,,	,,

Result of Inoculations of Blood on to Petri's dishes.

Amount of blood used.	Amount of medium used.	Result.
0·1 c.c.	10 c.c.	Sterile.
,,	,,	,,
,,	,,	Contaminated.

On August 19, 1904, this man's blood had given 316 micrococci per cubic centimetre, *vide* Experiment XI.

Table showing the Average Number of *M. melitensis* per cubic centimetres of Blood and the Day of Disease.

Experiment.	Day of disease.	Number of micrococci per cubic centimetres of blood.
I	84	2·6
II	17	10
III	14	100
IV	25	1·0
V	23	0·0
VI	25	3·3
VII	40	2·9
VIII	20	0·6
IX	3	3·3
X	10	0·0
XI	5	316·6
XII	24	0·0
XIII	13	0·0

[*Remarks.*—It is evident from Staff-Surgeon Gilmour's experiments that the *M. melitensis* is present in the majority of the cases examined. Their number is, however, so small that it seems extremely doubtful if this disease can be carried by biting insects.—Ed.]

Inoculation Experiments on Monkeys with Micro-organisms supposed to be *M. melitensis*, from the Blood of Patients suffering from Mediterranean Fever.

Experiment I.

A small, healthy, female Rangoon monkey, which had been under observation for 20 days. It had gone up in weight ½ lb., its coat had improved, and it appeared in perfect health. The temperature varied between 99°·6 and 101°·8. Its serum did not agglutinate *M. melitensis* in a dilution of 1—10. Weight 4 lbs. 12 ozs.

The object of this experiment was to prove that the coccus, obtained from the peripheral blood of a patient (W. A., age 32), was the *M. melitensis*.

October 6, 1903. This monkey was inoculated between the shoulder blades with an emulsion made from the contents of two sloped agar tubes (third generation of micrococcus) in 1 c.c. of broth.

October 7, 1903. Weight 4 lbs. 12 ozs.; appears well.

October 8, 1903. Weight 4 lbs. 10 ozs.; eating well.

October 9, 1903. Weight 4 lbs. 10 ozs.; seedy.

October 11, 1903. Weight 4 lbs. 5 ozs.; irritable, in other respects appears well. Its serum gives an immediate reaction to *M. melitensis* 1—10, 1—50, and 1—100 after 24 hours.

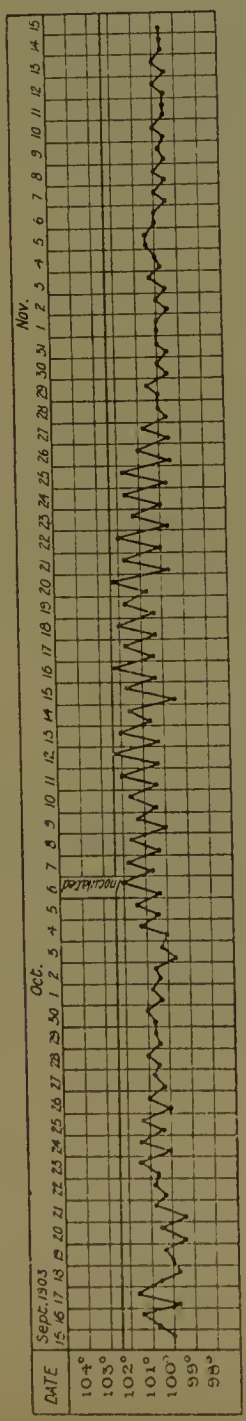
October 15, 1903. Weight 4 lbs. 2 ozs.; good reaction 1—100; seedy, but not very ill.

October 20, 1903. The monkey is improving in health. Slight reaction 1—50; good reaction, 1—30.

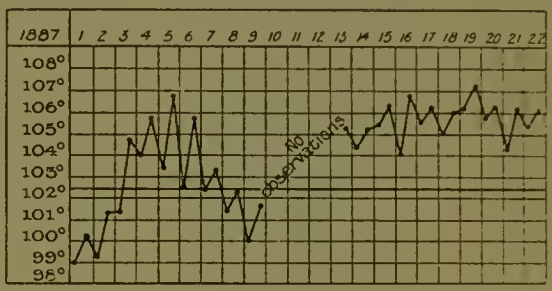
November 1, 1903. The animal has regained its weight and now weighs 4 lbs. 12 ozs. Perfectly well; reaction 1—10.

June 10, 1904. This monkey still reacts 1—10. It had no relapse.

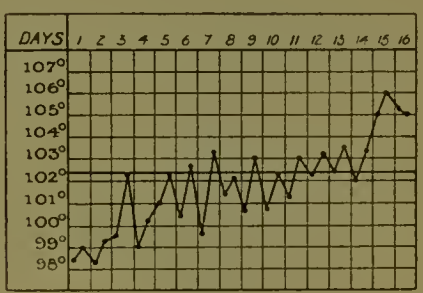
The following chart represents the temperature curve. Taken in the axilla.



[*Remarks.*—The temperature seems to have been taken in the axilla. It ought, in my opinion, to be taken in the rectum, the thermometer should be introduced as far into the intestine as possible, and a minimum of 5 minutes used for the observation. It is difficult to believe that this monkey can have had Malta Fever. The temperature chart shows no signs of the disease. Compare the following charts :—

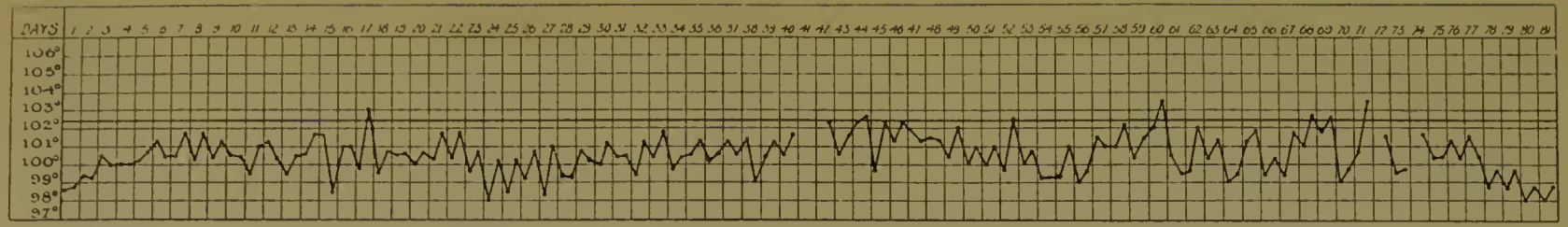


Monkey ♂. *Macacus rhesus.* Bruce. Temperature taken in the Axilla. Growth from Spleen.

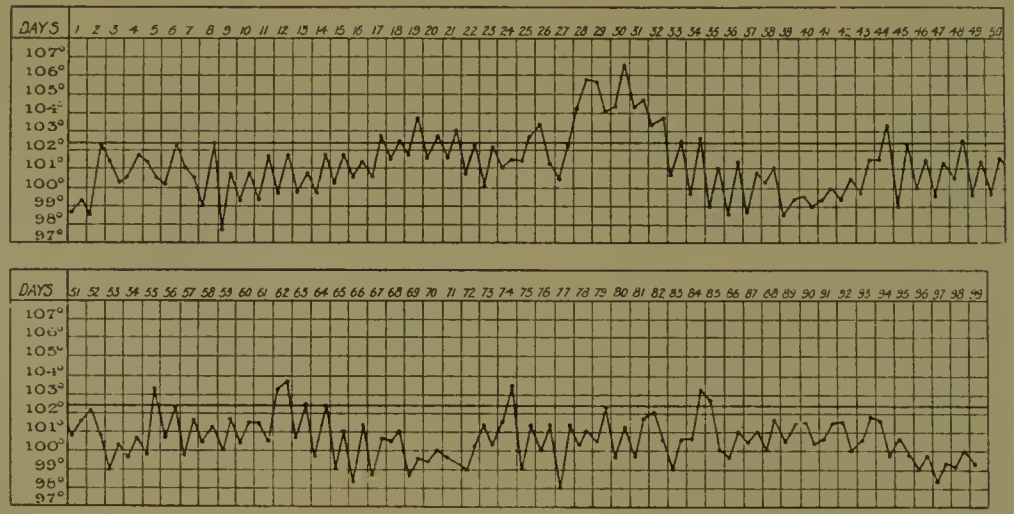


Monkey ♂. *M. rhesus.* Hughes. Axilla Temperature. Growth from Spleen.

Compare also the charts, Experiments V, VI, and XI Horrocks. In these cases the *M. melitensis* was recovered from the spleen after death. All these charts show a definite febrile disturbance, which is almost absent in the chart under consideration. It is certainly desirable that in these cases, the animal should be killed and the *M. melitensis* looked for in the spleen. Of course there is always the danger that the taking of the animal's temperature is entrusted to an ignorant or untrustworthy assistant.—Ed.]



Monkey ♂. Hughes. Axilla. Growth from Heart's Blood of Monkey.



Monkey ♀. *M. rhesus.* Hughes. Axilla. Growth from Spleen of Monkey.

Experiment II.

A small, healthy, male monkey, which had been kept under observation for 28 days. Weight 4 lbs. 9 ozs. No reaction 1—10.

November 16, 1903. This monkey was inoculated into the extensor muscles of the left thigh with 1 c.c. of an emulsion, made from three tubes of *M. melitensis* (first generation) in 2 c.c. of broth.

This experiment was carried out to prove that the growth, obtained from the peripheral blood of G. F., was the *M. melitensis*.

November 24, 1903. The monkey appears perfectly well. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400.

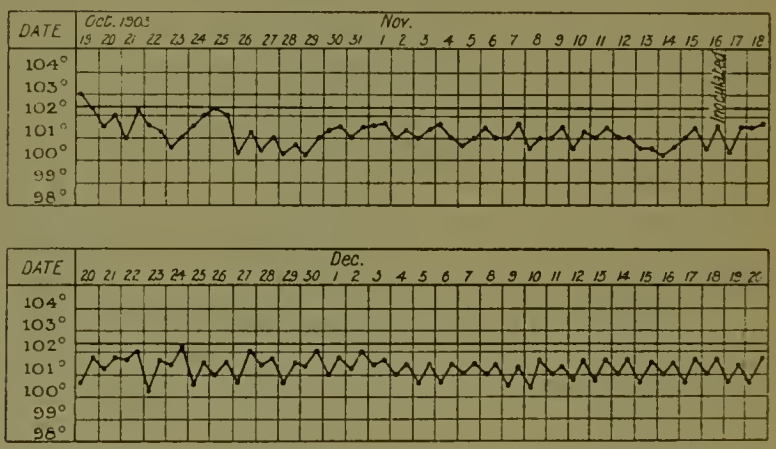
November 27, 1903. Weight 4 lbs. 8 ozs. Immediate agglutination reaction 1—400. The monkey was given a second injection of the contents of one tube, from same patient, into the muscles of the right thigh.

December 8, 1903. The monkey has remained perfectly well. Weight 4 lbs. 8 ozs. Agglutination reaction 1—200.

January 3, 1904. Monkey in good health. Agglutination reaction 1—200.

June 10, 1904. This monkey still reacts 1—10.

The following chart represents the temperature, taken in the axilla:



[*Remarks.*—This is also a very unsatisfactory temperature chart. The high agglutination reaction is, however, a strong argument that Staff-Surgeon Gilmour is dealing with *M. melitensis*.—Ed.]

Experiment III.

The following experiment was carried out to prove that the coccus, obtained from the knee-joint of F. B., age 21, was the *M. melitensis*.

Deeember 5, 1903. A male monkey, which had been under observation for a week, was inoculated into the extensor muscles of the left

thigh with an emulsion made from one tube (fourth generation) in 1 c.c. of sterile broth. It weighed 7 lbs. 6 ozs.; its serum would not agglutinate the laboratory *M. melitensis;* and its temperature was steady, 100° F.—100°·6 F.

The monkey remained well until December 8, the 3rd day after inoculation, when it shivered a good deal, went off its feed, and suffered from a rise of temperature, 102° F., in the evening.

After this date the monkey became very sick; its serum gave a negative reaction 1—10 on December 8; reacted 1—1200 on December 13, 1—1200 on the 17, and 1—3000 on the 20, the 15th day after inoculation; its weight decreased 1 lb. 4 ozs. by December 22, and its temperature remained up after the 3rd day, ranging between 101° and 102°·8 F.

December 23, 1903. The monkey was killed with chloroform, and a *post-mortem* held.

The organs were healthy, with the exception of the liver and spleen, which were congested. There were no signs of tubercle. Two sloped agar and two broth tubes were inoculated from the liver, three agar and two broth from the spleen, two agar from the heart's blood, and 30 c.c. of broth with 1 c.c. of heart's blood.

December 29, 1903. The tubes from the liver remained sterile and were destroyed.

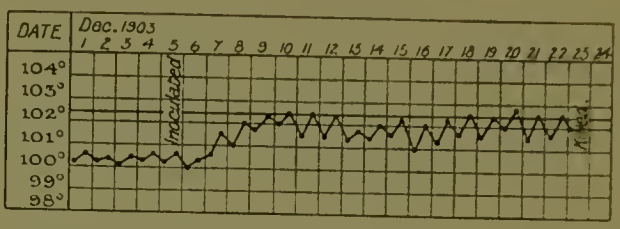
The agar tubes from the spleen showed no growth until the 3rd day, December 26, when many small isolated colonies appeared which, by the 4th day, had the appearance of a growth of *M. melitensis.* One broth tube from the same organ gave a growth by the 5th day; the other was sterile. A few transparent, isolated colonies also appeared on one agar tube from heart's blood on the 4th day.

The broth inoculated with heart's blood remained clear until the 3rd day, when it became slightly cloudy, after which the coccus grew rapidly; each field under the microscope being full of cocci. Sloped agar tubes (1 and 2), inoculated from the blood broth on December 24 and 27 respectively, remained sterile and were destroyed on December 29 and January 2. Two other tubes (3 and 4), inoculated on December 28, showed growth on December 31—isolated, transparent colonies, which the next day had every appearance of *M. melitensis.*

The tubes from the blood and spleen were examined microscopically, etc., and the growth—a micrococcus—was found to be identical in size, shape, motility, and staining reactions with the laboratory *M. melitensis;* it also gave an immediate agglutination reaction with the sera of the following Mediterranean fever patients: A. 1—500, B. 1—1000, P. 1—100, but not with healthy serum.

I think that the above experiments prove conclusively that the coccus, obtained in the first place from the synovial fluid of the knee-joint, was the *M. melitensis.*

The following chart shows the temperature curve :



[*Remarks.*—There can be little doubt that in this case Staff-Surgeon Gilmour is dealing with the *M. melitensis*. There is a distinct rise of temperature and the micro-organism was recovered from the spleen and blood.—Ed.]

6.

ISOLATION OF THE *MICROCOCCUS MELITENSIS* FROM THE BLOOD.

By Dr. T. Zammit, Member Mediterranean Fever Commission.

The patients of the Civil Central Hospital furnished, for the most part, the material for this investigation. The Honourable the Comptroller of the Charitable Institutions and the Medical Officers connected with that hospital deserve the thanks of the Commission for having kindly allowed the investigation to be conducted in the hospital.

The method followed at first was the simple one of drawing blood with a syringe from a vein at the bend of the arm. One to five cubic centimetres of blood was drawn, with all necessary precautions, and diluted in broth in the proportion of 1 of blood to 19 of broth. A proportion of 1 to 9 of broth was tried, but found unsuitable.

As soon as the blood was mixed with the broth it was taken to the laboratory where it was put in various proportions in 10 c.c. broth tubes and incubated. From the first mixture of blood 1, 2, 3, 4, 5 c.c., etc., were added to broth tubes and the dilution noted.

After an incubation of 4—5 days, a loopful of broth was passed over a sloped agar tube. When after 5 days no growth appeared on the agar, the same tube was reinoculated from the corresponding tube of broth, and so on every 5 days up to 1 month.

If a growth appeared having the appearance of the *M. melitensis* a note was made and the tube set aside for identification; if numerous

foreign growths appeared, the tube was usually thrown away and a note made that it was contaminated.

In some cases, however, the *M. melitensis* could be easily recognised among a lot of contaminations, and then sub-cultures were made to get a pure culture of the Micrococcus.

The contaminations observed during this investigation were traced to the imperfect preparation of the skin before drawing the blood, and, in fact, the contaminations were reduced to a minimum when a pad with carbolic solution (5 per cent.) was kept on the part for a few hours previous to the operation.

No bad effects were ever observed after the puncture, and no complaints were ever made by the patients.

After some time a few cases were met with in which, owing either to the prostrate condition of the patient or to his excessive nervousness the drawing of the blood from a vein by means of a syringe was not found to be possible. I, therefore, devised the following method of taking the blood which has proved so successful that I resorted to it constantly afterwards :—

The finger or the lobe of the ear of the patient is washed well with ether, ether-soap, water, alcohol and ether, and on the dry skin a puncture is made with a small syringe needle. With a sterile cotton wool pad the first drop of blood is removed, and an assistant squeezes the part for the next drop at the request of the operator. In a test-tube a large number of capillary tubes 1 cm. long are sterilised by dry heat, and at the time of collecting the blood, one of these short tubes is taken with fine forceps passed, immediately before, through the flame. As soon as the assistant squeezes the part and removes the cotton-wool pad, the tube is brought in contact with the drop, and when full is immediately put in a broth tube. This operation is repeated as long as the blood continues to ooze ; six tubes are usually filled. From these broth tubes, marked and incubated, passages on agar are made in the usual manner.

When a growth of *M. melitensis* is obtained on the agar slope, the capillary tube is drawn out of the broth, washed, dried, and weighed. It is then weighed again full of distilled water, and the difference between the two weights gives the volume of liquid the tube can hold, thus establishing to a nicety the amount of blood from which the *M. melitensis* has been isolated. By this method a volume of 0·005 of 1 c.c. of blood has been easily and accurately measured.

This method was used in twenty-two cases out of fifty with good results. Greater care is, of course, required in the disinfection of the skin, but when this extra trouble is taken the results compare most favourably with the bleeding from a vein. This method has also the great advantage that it can be applied to animals, as in case No. 50 in Table A.

Table A.

Order number.	Name and surname.	Sex.	Age.	Date of illness.	Character of case.	Temperature of body at time of experiment.	Amount of blood taken.	Minimum amount of blood in which *M. melitensis* was found.	Date of observation.	Remarks.
				Day.		°	c.c.	c.c.	1904.	
1	Giorgio Abdilla	M	40	25	Mild	99·8	2	—	June 21	No growth whatever.
2	Paolo Spiteri	M	49	100	,,	99·0	1	—	,,	,, ,,
3	Emmanuele Caruana	M	24	65	,,	99·8	1	0·1	June 23	
4	Ursola Vassallo	F	56	20	,,	101·0	1	0·1	,,	
5	Salvatore Camilleri	M	28	120	,,	100·0	1	—	June 27	,, ,,
6	Maria Chelcuti	F	29	13	Acute	100·2	1	0·2	,,	
7	Carmela Dimech	F	18	35	Mild	101·0	1	0·1	July 7	
8	Giuseppe Cordina	M	31	240	,,	99·8	1	—	,,	,, ,,
9	Alfredo Scicluna	M	38	14	Acute	102·0	1	0·1	July 8	
10	Pasquale Cachia	M	33	8	Mild	99·0	1	—	,,	,, ,,
11	Francesco Saliba	M	45	15	Acute	100·0	1	0·1	July 11	
12	Salvatore Ungaro	M	25	30	Mild	99·1	1	—	,,	,, ,,
13	Antonia Hili	F	23	8	Acute	104·4	few drops	0·02	July 15	
14	Luigia Brina	F	45	13	,,	103·2	,,	0·02	,,	
15	Carmelo Camilleri	M	21	30	Mild	103·2	1	—	,,	Tubes contaminated.
16	Giuseppe Farrugia	M	36	14	,,	101·0	1	—	,,	No growth whatever.
17	Mosè Azopardi	M	29	10	Acute	103·4	1	0·1	July 18	
18	Luigia Brina	F	45	16	,,	102·0	1	0·1	,,	
19	Raffaele Mercieca	M	15	7	,,	105·4	1	0·1	July 22	
20	Carmelo Fava	M	24	13	Mild	102·4	1	—	,,	Tubes contaminated.
21	Simeone Cumbo	M	30	15	,,	101·4	1	0·2	,,	
22	Giuseppe Micallef	M	39	18	,,	102·4	1	0·1	,,	
23	Caterina Pons	F	44	54	,,	102·0	1	0·1	July 27	

24	Angelo Ingnanez	M	37	12	Acute	102·0	1	0·5	,,		
25	Nattar Bassar	M	22	7	,,	101·0	1	0·1	,,		
26	Marianne Grima	F	24	32	Mild	101·0	1	0·1	,,		
27	Vincenzo Mamo	M	27	22	,,	101·0	few drops	—	,,	,,	,,
28	Salvatore Bonanno	M	46	13	,,	101·0	,,	—	,,	,,	,,
29	Carmelo Vella	M	26	150	,,	99·0	,,	—	,,	,,	,,
30	Patrick Bourke	M	33	65	,,	99·4	,,	—	,,	,,	,,
31	Giovanni Buhagiar	M	29	6	Acute	102·0	,,	—	Aug. 4	,,	,,
32	Carmelo Micallef	M	15	33	Mild	99·0	,,	—	,,	,,	,,
33	Giuseppe Zammit	F	17	7	Acute	104·0	,,	0·0097	Aug. 9		
34	Gio. Maria Mifsud	M	55	10	,,	101·0	1	0·1	Aug. 10		
35	Carmela Zammit	F	17	8	,,	103·8	1	0·025	,,		
36	Maria Teresa Perini	F	22	21	Mild	102·0	few drops	—	Aug. 11	No growth whatever.	
37	Maria Anna Fenech	F	25	30	,,	102·0	,,	—	,,	,,	,,
38	Nicola Farrugia	M	48	18	,,	100·6	1	—	Aug. 12	,,	,,
39	Taneredi Piacentini	M	31	7	Acute	102·2	1	0·025	,,		
40	Carmelo Grech	M	43	35	Mild	102·4	1	0·05	,,		
41	S. Valder	F	25	7	Acute	104·0	few drops	0·005	Aug. 17		
42	Carmela Bugeja	F	27	17	,,	105·0	,,	0·009	,,		
43	Giuseppa Grima	F	43	60	,,	106·0	,,	0·008	,,		
44	Anna Zammit	F	40	120	Mild	103·0	,,	—	Aug. 22	,,	,,
45	Jos. Sullivan	M	6	14	Acute	102·4	,,	—	Aug. 24	Tubes contaminated.	
46	Carmelo Delicata	M	56	18	Mild	99·0	,,	—	Aug. 25	,,	,,
47	Vincenzo Abela	M	32	8	,,	101·0	,,	—	,,	No growth whatever.	
48	Gaetano Billion	M	21	6	Acute	103·0	,,	0·006	,,		
49	Nicola Farrugia	M	48	31	Mild	103·0	,,	—	,,	,,	,,
50	Monkey No. 63	M	—	16	Acute	105·0	,,	0·005	Aug. 27		

The examination of fifty cases, made between June 21 and August 27, show that the *M. melitensis* circulates freely in the blood during an attack of fever, and that the amount of Micrococci varies usually with the temperature of the body.

In the fifty cases tabulated the *M. melitensis* was never recovered when the body temperature was below 100° F. At 102° and over it was recovered with the exception of two cases (Nos. 36 and 37), in which the tubes remained sterile, and in four cases in which the tubes were hopelessly contaminated. From one of these cases (No. 15) the *M. melitensis* was isolated by one of my colleagues on the same day.

Attempt to infect a monkey by means of a mosquito which had previously fed on a Mediterranean Fever patient.

Several mosquitoes (*Stegomyia fasciata*), which had previously been fed on an infected monkey (No. 45), were made to bite two healthy monkeys. No positive results were obtained. A positive result was obtained on the third attempt.

The third monkey (No. 63) was bought in Malta, along with two others, from a ship coming from the East Indies. Its temperature was taken twice daily after July 18, and it kept always within normal limits up to August 15.

The monkey was kept on the terrace on a side facing south-east, along with seven other animals, none of which had ever been ill.

On July 27 the blood of this monkey was tested, and it did not react to *M. melitensis* when diluted to 1 in 10.

On August 10 at 11 A.M. the monkey was bitten by two *Stegomyias* which had been fed at 11 A.M. on August 8 on a patient affected with a sharp relapse of Mediterranean Fever at the Civil Hospital (patient P. Sillato, Bed No. 40).

On August 20 the monkey was bitten again by one of the two *Stegomyias* used on the 10th.

On August 23 (13 days after inoculation) a rise of temperature was observed, and the blood of the animal was tested for Mediterranean Fever reaction, but no clear reaction could be obtained.

On August 26 the temperature rose again, and on the blood being tested, it was observed that it reacted strongly to *M. melitensis*. An immediate and complete agglutination was obtained at various dilutions up to 1 in 300. No further dilutions were tried.

The animal had obviously a sharp attack of fever, but the isolation of the coccus from the blood was necessary to make sure of the disease.

Without killing the animal, on August 31 one of its ears was properly disinfected and blood was drawn by pricking a small vein. The

blood was collected in small capillary pipettes 1 cm. long, in the manner described in another part of the Report, and put in broth.

On September 1 passages on agar were made from the broth tubes, and on the 4th a distinct growth was observed in one of the tubes. On the 5th two other tubes were found to have grown the Coccus.

All the growths tested in the ordinary way showed that the microbe was the *M. melitensis* in pure culture.

The least amount of blood from which the *M. melitensis* was obtained in this case was 0·005 c.c. Smaller quantities were not tried.

The position of the other monkeys, both healthy and ill, at the time of the experiment, is shown in the plan (p. 42). It is easily seen that no infected monkeys were anywhere near No. 63, and, therefore, direct infection from the monkeys, then ill on the same terrace, is highly improbable.

Experiments made in Malta by Dr. Zammit before the Appointment of the Commission.

1. *To Test Vitality of* M. melitensis *on Filter-paper exposed to Diffused Light.*

August	27, 1903.	A strip of filter-paper was hung on a wire inside a test-tube plugged with cotton-wool and sterilised by dry heat.
,,	28, ,,	Strip of filter-paper smeared with loopful of agar culture. Twelve tubes prepared in the same manner.
September	1, ,,	The filter-paper dropped in a broth tube and incubated. Growth obtained in due time.
,,	2, ,,	,, ,, Same result.
,,	3, ,,	,, ,, ,,
,,	4, ,,	,, ,, No growth obtained.
,,	5, ,,	,, ,, ,, ,,
,,	6, ,,	,, ,, ,, ,,

Conclusion.—*M. melitensis* retained its vitality for 7 days in diffused light. This experiment was repeated three times with the same result.

2. *To Test Vitality of* M. melitensis *in various Coloured Lights.*

Agar tubes inoculated with a drop of broth culture were incubated in cardboard boxes, of which the cover was made of a coloured glass plate. Violet, red, green, yellow, and blue plates were used. One tube was left in diffused light, and another one was wrapped in black paper.

Result.—No difference in growth was observed in the different tubes. The experiment was repeated three times with the same result, the tube exposed to blue light showing once a richer growth than the rest.

3. *Action of Direct Sunlight on Growth of* M. melitensis *in Agar Tube.*

September 17, 1903. Agar tube inoculated with 1 drop of broth culture was exposed for 15 minutes to the direct action of sunlight at about noon. Control tubes left in diffused light. No growth appeared before the 3rd day, but on the 4th day a growth was seen which in a few days was much more luxuriant than that on control tubes.

The experiment was repeated twice with the same result.

4. *Vitality of* M. melitensis *on Ordinary Limestone.*

September 12, 1903. Small bits of ordinary white porous limestone were taken and thoroughly sterilised. Emulsion made of *M. melitensis* from agar in sterile distilled water and the bits of stone wetted with this. The whole was kept in a dry atmosphere. On the 3rd day bits of the stone were dropped in broth tubes.

As former experiments had shown that light favours the growth of the *M. melitensis*, part of the bits of stone wetted with *M. melitensis* emulsion was kept in diffused light and part in a tube wrapped in thick black paper. The other conditions of the two tubes with pieces of stone were the same.

The result of the experiment was as follows:—

	Stone kept in dark.	Stone kept in diffused light.
Sept. 15 (3rd day).	Growth of *M. melitensis.*	Growth of *M. melitensis.*
„ 18 (6th „).	„ „	„ „
„ 19 (7th „).	„ „	„ „
„ 20 (8th „).	„ „	„ „
„ 26 (14th „).	No growth.	„ „
Oct. 28 (46th „).	„	„ „
Nov. 2 (51st „).	„	„ „
„ 19 (68th „).	„	No growth.

Conclusion.—Vitality of *M. melitensis* on limestone, in the dark, from 8 to 14 days.

Vitality of *M. melitensis* on limestone, in diffused light, not less than 51 days.

The experiment was repeated three times with practically the same result.

5. *To Test the Action of* M. melitensis *on the Reaction of Media.*

September 22, 1903. Seventy cubic centimetres of peptone broth with a reaction of +6, Eyre's scale, inoculated with loopful of *M. melitensis* from agar, and incubated at 37° C.

„ 26, „ Acidity reduced to +2.

October 28, „ Broth distinctly alkaline.

6. October 29, 1903. A series of test-tubes with 20 c.c. of broth in each were inoculated with a loopful of agar culture of *M. melitensis*. The tubes were then placed in large Buchner tubes half full with water and lightly covered so as to reduce the evaporation to a minimum. The whole was then incubated at 37° C. Tubes with broth were put for control in the same conditions.

November 19, „ (20th day). Acidity of broth + 2.
January 21, 1904 (82nd „). Broth alkaline − 3.
February 18, „ (110th „). „ − 4·5.

The control tubes showed an increased acidity. On the 20th day the acidity in the control tubes had doubled.

(This experiment is being repeated.)

7.

INTERIM REPORT OF EXPERIMENTAL WORK IN THE INVESTIGATION OF MEDITERRANEAN FEVER DEALING WITH BLOOD, SKIN, SWEAT, FILTRATIONS, AGGLUTINATING SERUM AND VARIOUS INOCULATIONS ON DIFFERENT ANIMALS.

By Staff-Surgeon E. A. Shaw, R.N., Member Mediterranean Fever Commission.

Examination of Blood.

The peripheral blood of Malta Fever patients has been examined by me for the *Micrococcus melitensis* (hereafter referred to as *M. melitensis*) in fifty-one cases, the results of which I append in a tabular form.

Method.—Bend of elbow prepared as for a surgical operation, blood withdrawn from median-basilic vein direct by means of carefully sterilised serum syringe.

½	c.c.	distributed over surface of agar in a Petri dish		A.
1	„	„	„	B.
2	„	„	„	C.
1	„	put into a 19 c.c. peptone broth tube	„	D.
1	„	„ another 19 c.c.	„	E.

ABCD kept intact, E used for making dilutions immediately, first well mixing blood and broth through a series of broth tubes by means of graduated pipettes sterilised in boiling water. At first the dilutions proceeded by multiples of 10; for instance, tube D contained 1 c.c. blood and 19 c.c. broth = a dilution of $\frac{1}{20}$, $2\frac{1}{2}$ c.c. of this contained $\frac{1}{8}$ c.c. blood and added to a 10 c.c. broth tube = $\frac{1}{8}$ c.c. of blood in $12\frac{1}{2}$ of mixture = a dilution of $\frac{1}{100}$; and abstracting 1 c.c. of this ($\frac{1}{100}$ c.c. of blood) and adding to a 9 c.c. broth tube = $\frac{1}{100}$ c.c. of blood in 10 of mixture = $\frac{1}{1000}$ dilution and so on up to $\frac{1}{100000}$.

All broth tubes and plates were duly labelled with a serial number for each patient, the quantity of blood contained, and the date and placed in the incubator at 37° C.

As time went on and the series of bloods increased it was found that *M. melitensis* was only being recovered from relatively large quantities of blood, up to Blood 15 never even from $\frac{1}{100}$ c.c. of blood and only occasionally from $\frac{1}{9}$ c.c., intermediate dilutions containing $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., and $\frac{1}{16}$ c.c. of blood were, therefore, made and incubated for Bloods 16, 17, 18, 19. The primary dilutions in Bloods 20 to 25 were made by multiples of 3 from the $\frac{1}{20}$ dilution, *i.e.*, $\frac{1}{60}$, $\frac{1}{180}$, $\frac{1}{540}$, $\frac{1}{1620}$, and $\frac{1}{4860}$. From Blood 26 onwards to Blood 51 by multiples of 2; thus one tube containing 19 c.c. of broth and one of blood remained as the unit 1 c.c. of blood, the other tube of similar contents had 10 c.c. abstracted and was hence left containing $\frac{1}{2}$ c.c. blood, the 10 c.c. removed was added to a 10 c.c. broth tube, the resulting 20 c.c. of mixture well amalgamated, and 10 c.c. then abstracted thus leaving it containing $\frac{1}{4}$ c.c. blood; and thus tubes containing $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, and $\frac{1}{256}$ c.c. respectively of blood were prepared, the intention being to increase these dilutions if *M. melitensis* was ever recovered from the highest, though the first twenty-five bloods drawn had not yielded it in so high a dilution as $\frac{1}{100}$.

These blood dilutions were daily thoroughly well shaken to give the *M. melitensis* an opportunity of emerging from the leucocyte in which it was thought to be most probably lodged, and after 5 days incubation, subcultures on to agar slopes from the respective broth tubes were prepared and incubated at 37° C. These inoculations of agar slopes were repeated when considered necessary and no blood dilution was abandoned as unfruitful till it had been incubating at least 11 days.

The Petri dish method was worked side by side with the broth enrichment method for the first seventeen cases, afterwards it was abandoned. The original idea was that the number of colonies of *M. melitensis* appearing could be taken as an index of the quantity of *M. melitensis* in the measured quantity of blood taken. It was found quite easy by inclining the plate to get the blood put on the agar surface to spread itself quite evenly over the whole area of agar forming a very thin layer, but when, as in Cases 10, 12, 14, and 16,

M. melitensis was recovered by the broth method, while the plate method failed to show it, time was felt to be too valuable to persevere with the latter.

Of the details given in the tabulated results some explanation is necessary. In the column headed nation and sex, E = English, M = Maltese, A = Army, N = Navy, F = Female, and as the only female patients from which blood was taken were Maltese, the sex is specified only for that nationality, thus M.M. = Maltese male, and M.F. = Maltese female. The English patients were all male.

The temperatures given preceding drawing of blood are for the few days immediately prior to drawing of blood, the last being the temperature on day of abstraction of blood, these are given as follows: $\frac{101}{103}$ the upper temperature being the morning the lower the evening temperature. In some of the Maltese cases where, owing to the frequent unexpected discharge of patients at their own request prompt action was necessary, blood was taken very soon after admission, and in such cases temperature for only 1 or 2 days could be so given.

The day of disease is enumerated from the first onset of symptoms attributable to the fever.

The time at which blood was drawn is given, it was noted with the intention of seeing if any difference in result would appear between blood taken in the forenoon and that taken in the evening, the patient's temperature at time of drawing is here given also.

The agglutination test was applied by me to all samples of blood drawn, to independently confirm the diagnosis of Malta Fever, and after working out eighteen bloods, it was felt it would be of interest to know the *limit dilution* which would agglutinate a standard fresh agar growth of *M. melitensis* to see if there was any relation between amount of *M. melitensis* obtained from a given blood and the agglutinating power of the latter. The standard taken is an arbitrary one, being that agglutination should be unmistakably marked under the $\frac{2}{3}$ inch objective, 15 minutes after the mixing of *M. melitensis* emulsion and diluted serum, invariably comparison was made with a control.

In the column headed Recovery of *M. melitensis* the sign + means recovery, and the sign − means no recovery.

Smallest quantity of blood means the smallest quantity calculated from the highest broth dilution yielding *M. melitensis* and the amount of blood therein contained.

The following tests were invariably applied to each recovery of *M. melitensis* before it was entered as such in the laboratory records:—

1. Growth on agar slope should be that characteristic of *M. melitensis*.

2. Size and appearance of cocci in film stained with dilute carbol-fuchsine should be characteristic.

No. of case.	Nation and sex.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood swing aggl.	Recovery of *M. melitensis.*	Smallest quantity of blood giving *M. melitensis.*
				° F.					
1	E. A.	37	Had 3 waves. Now convalescent	Normal for preceding 20 days	98th	12.30 noon, N.	Aggl.	+	$\frac{1}{2}$ c.c.
2	„	31	Had 1 wave	Normal for preceding 7 days	30th	12.30 noon, N.	Aggl.	–	
3	„	28	T. never normal since admitted; a long severe case	E.T.'s = 101, 100, 99, 99.....	101st	12.30 noon, N.	Aggl.	–	
4	„	31	Mild case, 4 waves ..	Normal for preceding 30 days.....	108th	12.30 noon, N.	Aggl.	–	
5	M. M.	40	End of 4th wave ..	99, $\frac{98}{98\cdot4}$, $\frac{99}{\quad}$	74th	Noon, N.	Aggl.	–	
6	„	22	In 1st wave	$\frac{101\cdot6}{102\cdot4}$, $\frac{101}{102\cdot6}$, $\frac{101}{\quad}$	15th	Noon, 100°·8	Aggl.	–	
7	„	24	End of 2nd wave..	$\frac{99\cdot4}{101}$, 99, 98, 98	49th	11.30 noon, N.	Aggl.	+	$\frac{9}{10}$ c.c.
8	M. F.	56	End of 2nd wave..	N., $\frac{N.}{99\cdot6}$, N., N.	30th	11.30 A.M., N.	Aggl.	+	$\frac{3}{10}$ c.c.
9	„	28	Nearing end of 2nd wave	99, $\frac{99}{101\cdot6}$, 99·6, $\frac{100\cdot4}{\quad}$	41st	Noon, 100°	Aggl.	–	
10	„	18	No information ...	N., 99, $\frac{98}{101\cdot6}$, $\frac{101}{\quad}$	37th	11.45 A.M., 100°·6	Aggl.	+	$\frac{1}{5}$ c.c.
11	M. M.	31	Now in hospital for orchitis. Had fever 8 months ago	Normal for months.....	240th	Noon, N.	Aggl. $\frac{1}{5}$	–	

12	„	38	In 1st wave	$\frac{100}{100}$, $\frac{99\cdot6}{101}$, $\frac{99}{100}$, $\frac{100}{102}$	10th	5.15 P.M., 102°	Aggl.	+	$\frac{1}{8}$ c.c.
13	„	30	In 1st wave	$\frac{98}{102\cdot5}$, $\frac{}{99}$	9th	5.30 P.M., 99°	Aggl.	−	
14	„	47	Ill at home 3 months. Now admitted because worse	$\frac{100}{102}$, $\frac{101}{101}$, $\frac{100}{101}$, $\frac{100}{99\cdot4}$	95th	5.0 P.M., 99°·4	Aggl.	+	$\frac{1}{9}$ c.c.
15	„	25	Nearing end of 1st wave	$\frac{101}{101}$, $\frac{101}{99\cdot2}$, $\frac{99\cdot2}{99\cdot2}$, $\frac{99\cdot1}{}$	31st	5.20 P.M., N.	Aggl.	−	
16	„	22	Middle of 3rd wave	$\frac{99}{103\cdot2}$, $\frac{99}{103\cdot2}$, $\frac{100\cdot4}{103\cdot2}$, $\frac{102}{102}$, $\frac{103\cdot6}{103\cdot2}$ ..	38th	5.10 P.M., 103°·2	$\frac{1}{1000}$	+	$\frac{1}{4}$ c.c.
17	„	36	In 1st wave	$\frac{}{100\cdot2}$, $\frac{101\cdot3}{101}$, $\frac{101}{103}$, $\frac{101\cdot1}{101}$, $\frac{101}{101\cdot6}$ ..	17th	5.25 P.M., 101°·6	Aggl. $\frac{1}{40}$	−	
18	„	29	In 1st wave	$\frac{102\cdot2}{102\cdot8}$, $\frac{}{103\cdot4}$	9th	5.30 P.M., 103°·4	$\frac{1}{40}$	+	$\frac{1}{9}$ c.c.
19	M. F.	44	In 1st wave	$\frac{102\cdot4}{103\cdot8}$, $\frac{103}{102\cdot4}$, $\frac{101}{103}$, $\frac{101\cdot2}{103}$, $\frac{101}{102}$ ..	15th	5.45 P.M., 100°·5	$\frac{1}{640}$	+	$\frac{1}{8}$ c.c.
20	E. N.	22	In 2nd wave....	$\frac{100}{103\cdot6}$, $\frac{100}{102\cdot6}$, $\frac{100}{102\cdot4}$, $\frac{99\cdot6}{102\cdot4}$, $\frac{100\cdot2}{}$	22nd	10.30 A.M., 100°	$\frac{1}{500}$	−	
21	„	32	In 1st wave	$\frac{101}{102\cdot4}$, $\frac{102\cdot2}{103}$, $\frac{97\cdot6}{99\cdot6}$, $\frac{N.}{100}$, $\frac{N.}{}$	28th	10.20 A.M., N.	$\frac{1}{1000}$	−	
22	M. M.	15	In 1st wave	$\frac{103\cdot6}{104\cdot2}$, $\frac{102}{105\cdot4}$	11th	5.30 P.M., 103°·8	$\frac{1}{300}$	+	$\frac{3}{4}$ c.c.
23	„	24	In 1st wave. Continuous fever	$\frac{101}{102\cdot4}$, $\frac{101}{102\cdot4}$	31st	5.40 P.M., 102°	$\frac{1}{300}$	+	$\frac{1}{6}$ c.c.
24	„	29	In 1st wave	$\frac{103}{103}$, $\frac{102}{102\cdot6}$, $\frac{101\cdot4}{102\cdot4}$, $\frac{101\cdot6}{101}$, $\frac{99\cdot8}{101\cdot4}$ ..	13th	5.50 P.M., 99°·4	$\frac{1}{1080}$	−	
25	M. F.	39	In 1st wave	$\frac{99\cdot4}{101}$, $\frac{99\cdot4}{101\cdot6}$, $\frac{100}{101\cdot4}$, $\frac{100}{101\cdot4}$, $\frac{100}{102\cdot4}$.	18th	6.10 P.M., 101°·4	$\frac{1}{1080}$	−	
26	M M.	37	In 1st wave	$\frac{100}{102\cdot4}$, $\frac{100\cdot8}{102}$, $\frac{98}{101}$, $\frac{101}{102}$	12th	5.0 P.M., 102°	$\frac{1}{1000}$	+	1 c.c.
27	„	22	In 1st wave	$\frac{}{100}$, $\frac{100}{101}$	7th	5.15. P.M., 102°	$\frac{1}{800}$	+	$\frac{1}{8}$ c.c.

No. of case.	Nation and sex.	Age.	Stage of the fever.	Temperature of patient for few days preceding bleeding.	Day of disease.	Time of bleeding and patient's temperature.	Maximum dilution of patient's blood swing aggl.	Recovery of *M. melitensis.*	Smallest quantity of blood giving *M. melitensis.*
				° F.					
28	M. F.	24	In 1st wave. Continuous fever	99·6 / 102·8 ; 101·2 / 103 ; 101 / 101·2 ; 99 / 101·8 ; 100 / 103·2	32nd	5.30 P.M., 102°	$\frac{1}{1200}$	−	
29	„	44	In 2nd wave.....	101 / 102·4 ; 99 / 102·6 ; 100·2 / 102 ; 100·2 / 101·6 ; 101·4 / 102	56th	5.45 P.M., 101°·8	$\frac{1}{1000}$	+	$\frac{1}{4}$ c.c.
30	E. A.	23	In 1st wave	101 / 102·3 ; 100·6 / 102·8 ; 100·6 / 102·8 ; 100·5 / 101·8 ; 100·4 / —	15th	11.0 A.M., 100°	$\frac{1}{500}$	+	$\frac{1}{16}$ c.c.
31	„	27	In 1st wave	100 / 101 ; 101·6 / 103 ; 101·6 / 103 ; 101·6 / 104·2 ; 102 /	22nd	11.15 A.M., 101°·8	$\frac{1}{800}$	−	
32	„	37	In 1st wave	99 / 101·6 ; 99·6 / 103·4 ; 101 / 102·6 ; 102 / 103 ; 99·4 /	36th	11.30 A.M., 99·8	$\frac{1}{2000}$	+	1 c.c.
33	M. M.	55	In 1st wave	100 / 100 ; 99·4 / 100·6 ; 99·2 / 100·6 ; 99·4 / 102·2 ; 101 / 101	10th	5.10 P.M., 101°	$\frac{1}{600}$	+	1 c.c.
34	„	17	In 1st wave	..... 103 / 104	8th	5.30 P.M., 103°·8	$\frac{1}{400}$	+	$\frac{1}{64}$ c.c.
35	„	38	In 1st wave	101 / 101 ; 101 / 101 ; 99·2 / 101 ; N. / 102 ; 99·2 / 100·6	18th	5.0 P.M., 100°·6	$\frac{1}{300}$	+	$\frac{1}{2}$ c.c.
36	„	31	In 1st wave	..... 101 / 103·8 ; 101·8 / 102·2	7th	5.10 P.M., 102°·2	$\frac{1}{40}$	+	$\frac{1}{128}$ c.c.
37	„	43	No information ...	..... 101·8 / 102·4	36th	5.20 P.M., 102°·4	$\frac{1}{1000}$	+	$\frac{1}{64}$ c.c.
38	E. A.	22	Middle of 2nd wave	102·6 / 103 ; 102·8 / 104 ; 101 / 103·8 ; 102 / 103·4 ; 102·6 / 102	26th	5.40 P.M., 102°	$\frac{1}{1000}$	+	$\frac{1}{16}$ c.c.

39	„	21	Near end of 1st wave	104 103 101 98·4 98·4 ..... / 104·2, 104, 102, 99·8, —	9th	11.0 A.M., 98·4	$\frac{1}{800}$	+	$\frac{1}{8}$ c.c.
40	„	22	Near end of 3rd wave	98·6 99·2 99·2 99·6 99·2 .. / 100·6, 100·6, 101, 100·6,	57th	11.10 A.M., 99°·2	$\frac{1}{1000}$	−	
41	„	38	Near end of 2nd wave	98·8 98·6 98·4 99 99 .... / 100·8, 100, 100·2, 100·2,	48th	11.20 A.M., 99°·4	$\frac{1}{1800}$	−	
42	„	32	Near end of 2nd wave	100·4 100·4 100·1 99·5 99·4 .. / 102·3, 102·7, 101·5, 102,	55th	11.30 A.M., 99°·6	$\frac{1}{600}$	+	$\frac{1}{6}$ c.c.
43	E. N.	26	In 1st wave	98 99 98·6 99·6 99·8 .... / 99·4, 99·4, 99·6, 100·6,	15th	10.30 A.M., 99°·6	$\frac{1}{800}$	+	$\frac{1}{250}$ c.c.
44	„	27	In 1st wave	99·2 99 97·6 98·6 98·6 .... / 100·4, 102, 101·4, 101,	17th	10.20 A.M., N.	$\frac{1}{1800}$	−	
45	E. A.	31	Nearing end of 2nd wave	98·6 98·4 98·5 98·4 98·8 ... / 100, 100·6, 100·2, 101,	41st	9.50 A.M., 99°	$\frac{1}{600}$	+	$\frac{1}{16}$ c.c.
46	„	27	Nearing end of 3rd wave	100 100 100 99·2 99 ..... / 101, 102·6, 101, 101,	69th	10.5 A.M., 99°	$\frac{1}{1400}$	−	
47	„	20	Middle of 1st wave	102·2 102 101·2 101 101 .... / 103·4, 103·4, 103·2, 103,	25th	11.10 A.M., 101°	$\frac{1}{1000}$	+	$\frac{1}{250}$ c.c.
48	„	20	Now commencing 2nd wave	99 101 101 102·4 101 .. / 101·6, 103·5, 103·4, 103·2,	28th	11.20 A.M., 101°	$\frac{1}{1800}$	−	
49	„	38	Height of 1st wave	100 102 102·8 101 102 .. / 103·6, 104·6, 104·7, 105·6,	22nd	11.30 A.M , 102°	$\frac{1}{1400}$	+	1 c.c.
50	„	20	Middle of 1st wave	102·2 100·9 101 100·4 100·6 / 103·2, 103·2, 102·7, 102·8,	42nd	10.50 A.M., 100°·4	$\frac{1}{600}$	+	$\frac{1}{125}$ c.c.
51	„	40	Only now nearing end of 1st wave	99·2 99 99 99·2 99 .... / 101, 100·4, 100·4, 100·5, —	55th	11.5 A.M., 99°·4	$\frac{1}{200}$	+	$\frac{1}{6}$ c.c.

3. Non-staining with Gram.

4. No development of gas, acidity or coagulation when grown in litmus milk, but production of alkalinity.

5. No production of acidity, but production of alkalinity when grown on glucose-litmus-agar.

6. Mobility in hanging drop merely Brownian, no translation from portion to portion of field.

7. Should be agglutinated, visibly to the naked eye by a $\frac{1}{500}$ dilution of a pure animal serum, obtained by inoculating an animal (rabbit and monkey were both used), with a pure standard growth of *M. melitensis*. Comparison with a control was always made, and the two submitted to my fellow-worker, Major Horrocks, R.A.M.C., at the next bench, and unless he concurred as to the indubitable nature of the reaction it was not accepted.

There has been considerable difficulty in extending this series of blood examinations even so far as it has gone. Patients did not like it; some consented freely, others reluctantly, and their physicians were not prepossessed in favour of it either. One would have liked to have taken a few cases and taken specimens of blood every day or every other day, and so ascertained when the *M. melitensis* appeared in and disappeared from the peripheral blood during the whole course of the fever; but it was found impossible to accomplish this. Only with one patient did I succeed in getting blood twice for examination; the first time reported as No. 6, result negative; and the second time as No. 16, result positive.

As regards syringes, I found it simplest to sterilise them in the antoclave at 120° C. The needles I found did best sterilised in pure olive oil at about 140° C.; this prevented rust and their points retained their primitive sharpness. I also found blood was obtained with greater facility if the needle were passed into the vein from the bend of the elbow towards the hand, so that blood entered the syringe in the direction of natural flow.

This method of taking blood from the median basilic vein and incubating it in broth was apparently first described by Dr. Jules Courmont, at a meeting of the Société Medicale des Hôpitaux de Paris, December 27, 1901, who applied it successfully in nine cases of Typhoid Fever in which he recovered *B. Typhosus* from the peripheral blood. I saw the method in application in Widal's Clinique in the Hôpital Cochin in Paris in the winter of 1902–3, there studied it and applied it successfully to the recovery of *M. melitensis* from the peripheral blood of Malta Fever patients in the summer of 1903. So far as I know the dilution method to determine the smallest quantity of fluid containing the micro-organism has not hitherto been applied in the recovery of micro-organisms from the circulating blood, though it is classical in the history of the bacterial analysis of water. It has

obvious advantages over the plating method, a most important one being that as in Blood No. 27, there were only nine growths representing the nine dilutions 1, $\frac{1}{2}$, $\frac{1}{4}$, $\frac{1}{8}$, $\frac{1}{16}$, $\frac{1}{32}$, $\frac{1}{64}$, $\frac{1}{128}$, $\frac{1}{256}$ c.c., to examine and put through the various tests for *M. melitensis*; whereas had 1 c.c. of this blood been plated out, it would presumably have yielded over 200 colonies, which would have required verification individually, as unfortunately all the colonies found in a blood plate are not necessarily of the same kind, and one cannot apply the principle *Ex uno omnes disce*.

Conclusions.

1. *M. melitensis* exists in the blood of patients in relatively very small amount, the smallest quantity of blood in which it has been found, $\frac{1}{256}$ c.c. is practically the equivalent of 4 c.mm. and as 1 cubic millimetre of blood = 5,000,000 corpuscles, and if *M. melitensis* is never found in association with less number of corpuscles than 20,000,000 it is obvious there is no comparison between this and such a disease as anthrax, for instance, in which in the blood the number of bacilli has been found in some cases equalling the number of corpuscles. This has an important bearing on the question of transmission of infection by mosquitoes.

2. No definite relation can be established between any given stage of the disease and the presence of *M. melitensis* in the blood. It has been found as early as the 7th day Cases 27 and 36, and as late as the 95th and 98th day Cases 1 and 14. It has been found in the majority of cases when the temperature of the patient has been raised, but it has been also present in convalescence (Case 1), and when temperature has been normal (Cases 7, 8 and 39), for several days, but it has also not been found when the temperature was high, Cases 6, 25, 28, 31, and 48.

3. There is some indication of a diurnal variation in its presence in the blood, out of 29 cases where blood was taken in the forenoon between the hours of 10 and 12.30, it was present in 14, absent in 15. Out of 22 cases where blood was drawn in the evening between 5 and 6.30 p.m. it was present in 16, absent in 6; a ratio of almost 3 : 1 in favour of the evening.

4. No relation can be established between the agglutinating power of a patient's blood for *M. melitensis* and the amount of the latter present in the blood, most of the cases in which it was found had a high agglutinating power, but one of the cases in which *M. melitensis* was found in one of the smallest quantities of blood, $\frac{1}{128}$ c.c. (Case 37) only agglutinated in a $\frac{1}{10}$ dilution, as against another in which it was found in $\frac{1}{256}$ c.c., in which there was agglutination with a dilution of 1 in 1000, and others where it was not found at all where there was agglutination in a dilution of $\frac{1}{1000}$, Cases 41, 44, and 48.

5. In some of the cases the *M. melitensis* was found to have skipped some of the dilutions, for instance, in Case 34, where the dilutions proceeded by powers of 2 from 1 to 256, *M. melitensis* was found in the 1 c.c., $\frac{1}{2}$ c.c., $\frac{1}{4}$ c.c., dilutions, absent from the $\frac{1}{8}$ c.c. and $\frac{1}{16}$ c.c. dilutions, present in the $\frac{1}{32}$ and $\frac{1}{64}$ dilutions, absent in the rest. In Blood 37, in which same series of dilutions were made, *M. melitensis* was present in all up to the $\frac{1}{64}$ c.c. inclusive, with the exception of the $\frac{1}{16}$, these were the only two cases out of the fifty-one in which this jumping took place. It is certainly not due to inadequate mixing of the dilutions, for the primary blood dilution, from the moment the blood got into it, which was instantly on the needle being withdrawn from the vein, was agitated vigorously until a considerable froth was on its surface, and so on with the succeeding dilutions. It may possibly be due to the small quantity of *M. melitensis* in the blood, or to the *M. melitensis* being in some dilutions so phagocyted as to be unable to escape and multiply.

Examination of Bloods.

Table showing in chronological order the date of the disease in each case in which blood was taken for bacteriological examination, and the result. The fractions of a cubic centimetre indicate the smallest amount of blood from which *M. melitensis* was obtained; the sign – means no *M. melitensis* was recovered; the days of disease which are not represented by a blood examination are shown blank. It will be seen that while many days are blank, others are represented by 1, 2, 3, or 4 examinations of blood. This has been unavoidable; th number of cases willing to submit to venous puncture was too small to admit of selection; and waiting a few days usually meant losing the case.

Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.	Day of disease.	Recovery and quantity or no recovery.
1		38		75	
2		39		76	
3		40		77	
4		41	−, $\frac{1}{10}$ c.c.	78	
5		42	$\frac{1}{128}$ c.c.	79	
6		43		80	
7	$\frac{1}{8}$, $\frac{1}{128}$ c.c.	44		81	
8	$\frac{1}{64}$ c.c.	45		82	
9	−, $\frac{1}{9}$, $\frac{1}{8}$ c.c.	46		83	
10	$\frac{1}{9}$, 1 c.c.	47		84	
11	$\frac{3}{4}$ c.c.	48	−	85	
12	1 c.c.	49	$\frac{9}{10}$ c.c.	86	
13	−	50		87	
14		51		88	
15	−, $\frac{1}{8}$, $\frac{1}{10}$, $\frac{1}{256}$ c.c.	52		89	
16		53		90	
17	−, −	54		91	
18	−, $\frac{1}{2}$ c.c.	55	$\frac{1}{64}$, $\frac{1}{64}$ c.c.	92	
19		56	$\frac{1}{1}$ c.c.	93	
20		57	−	94	
21		58		95	$\frac{1}{9}$ c.c.
22	−, −, 1 c.c.	59		96	
23		60		97	
24		61		98	$\frac{1}{2}$ c.c.
25	$\frac{1}{256}$ c.c.	62		99	
26	$\frac{1}{10}$ c.c.	63		100	
27		64		101	−
28	−, −	65		102	
29		66		103	
30	−, $\frac{9}{10}$ c.c.	67		104	
31	−, $\frac{1}{6}$ c.c.	68		105	
32	−	69	−	106	
33		70		107	
34	$\frac{1}{4}$ c.c.	71		108	−
35		72		240	−
36	1, $\frac{1}{64}$ c.c.	73			
37	$\frac{7}{8}$ c.c.	74	−		

Examination of Epidermis of Malta Fever Patients for M. melitensis.

Method.—Patients were selected with temperatures of 100° F. and upwards in different stages of the fever from the 15th to 60th day, epidermis from the arms and flanks scraped away with a sharp sterilised scalpel till the dermis threatened pin-point hæmorrhages, the scrapings put in sterilised capsules, taken to the laboratory and there ground up in a small quantity of sterile normal salt solution −(1 c.c.). From this three successive agar Petris were inoculated with one loopful, to the remainder, 5 c.c. of salt solution was added, and the surface of three other agar Petris inoculated by spreading $\frac{1}{4}$ c.c. of this diluted skin emulsion over each, and the whole incubated at 37° C. for 5 days.

Up to the present this method has been applied to twelve cases.

Discrete colonies of the different micro-organisms usually met with in the skin were obtained in every case, but in none of these plates were colonies of *M. melitensis* ever obtained.

Examination of Sweat from Malta Fever Patients for M. melitensis.

1*st Method.*—A skin surface of forearm washed with spirit soap, then ether, a carbolic pad 1 in 40 kept on 12 hours, then a circle of sterilised (dry 160° C. air) lint placed on this surface, and a sterilised watch glass strapped over it with adhesive plaster. After critical sweating, circle of lint removed, placed between two sterilised watch glasses held in a metal frame, and sent to me at laboratory. There each circle of lint placed in a separate broth tube numbered, dated, and incubated at 37° C. After 5 days' incubation, agar slopes, inoculated zig-zag from each, incubated at 37° C. and examined daily for growth; if sterile, original broth tubes were inoculated with *M. melitensis* returned to incubator for 4 days and then fresh slopes inoculated from them; on these *M. melitensis* invariably appeared, thus proving that sufficient disinfectant to prevent growth of *M. melitensis* had not been carried into circles of lint from disinfection of skin surface. Nineteen sweat swabs from different patients were thus examined. In some cases the tubes remained sterile, in others the agar slopes yielded growth in discrete colonies.

Result.—No *M. melitensis* was ever recovered by this method.

2*nd Method.*—The critical sweat was collected in sterile pipettes from four different patients, zig-zagged on agar and incubated. The collection was done by the sisters in the ward who were supplied with the pipettes ready for use, and instructed how to break off the points and apply them. They stated it was rare for sweat to collect in such large drops as to admit of collection in this manner, hence specimens were obtained from only four patients.

Result.—No *M. melitensis* was obtained.

3*rd Method.*—(A modification of the 1st.)—Circles of lint were obtained saturated with critical sweat from Malta Fever patients as in 1st Method, but instead of being incubated in broth tubes were placed each in a 5 c.c. sterile normal salt solution tubes, in which they were thoroughly agitated and ground up with a sterile glass rod, and the resulting fluid plated out in agar Petri dishes both by spreading $\frac{1}{2}$ c.c. of it over whole surface, and by describing a centripetal spiral with a loop full of the fluid. Discrete colonies were always thus obtained after incubation at 37° C.

The critical sweats of seven patients have been thus examined without *M. melitensis* having been obtained.

To see if M. melitensis *would Pass any Filter.*

It was felt it would be of the greatest assistance in isolating *M. melitensis* if advantage could be taken of its small size to separate it from other larger organisms by means of filtration, and I, therefore, experimented with the following filters as described :—

New filters were used for the first time in each case. Obviously the first indication was to find a filter that would pass *M. melitensis* and later to see if *M. melitensis* would come through it from a mixture of microbes. Bougies were all first tested for imperfections by placing in water and applying air under pressure. *Chamberland F.* was first tried after being sterilised in the autoclave at 155° C. 1 hour. All junctions were luted with paraffin.

July 8. Placed broth emulsion of verified living *M. melitensis* from one agar slope in container, filled up with peptone broth, tightened pinch cock, placed apparatus in incubator at 37° C.

July 9. Broth in flask remains clear, loosened pinch cock and ran in 6 drops from bougie. This was repeated daily till July 30, apparatus being kept in incubator at 37° C. all the time.

July 30. Three agar slopes inoculated with some of filtrate, drawn off with a sterile pipette from flask through side tube and incubated.

August 4. No growth in any of agar slopes. Experiment concluded.

Result.—No *M. melitensis* has either been washed through or has grown through Chamberland F.

2nd Filtration Experiment with M. melitensis.

July 7. Took Chamberland F. bougie, tested for imperfections in water with air under pressure, cut off porcelain end, heated resulting cylinder to redness in moufle ; fitted up to act as filter, first sterilising all glass parts at 180° C. for 30 minutes, then sterilised apparatus in autoclave 30 minutes at 120° C., and finally luted junctions with paraffin.

July 8. Placed emulsion of living tested *M. melitensis* (emulsion in broth from growth on one agar slope) in cavity of bougie and filled up with peptone broth, removing glass rod in rubber cork to allow of escape of contained air ; replaced plug of wool in end of tube ; replaced glass rod ; and placed apparatus in incubator at 37° C.

July 9. Broth coming through filter into cavity of test-tube, displaced air escaping by tube B which had been also plugged with cotton wool.

July 27. Apparatus has now been in incubator 18 days. Inoculated three agar slopes with filtrate obtained by means of a sterile pipette passed down tube B, and placed these in incubator at 37° C.

July 31. Agar slopes have now been in incubator 4 days and remain without growth.

Result.—M. melitensis does not pass Chamberland F.

3rd Filtration Experiment with M. melitensis.

To see whether *M. melitensis* will *pass* any of three Berkefeld filters N., V., and W., of differing porosities (these were obtained from the Lister Institute).

One of each porosity was taken, tested in water with compressed air, sterilised, and fitted up, glass container being first sterilised by boiling in water and then in hot air 1 hour at 160° C. An air pass being arranged in rubber collar to allow of air displaced by filtrate escaping from container. Then the whole sterilised in autoclave at 115° C. for ½ hour.

August 7. Eight cubic centimetres of 5 days' old verified broth growth of *M. melitensis* placed in each bougie with a sterile pipette.

August 8. Some filtrate in container, 8 c.c. more of same broth culture placed in each bougie.

August 9. Five cubic centimetres more of same culture in each bougie.

August 10. Five cubic centimetres more of same culture in each bougie.

August 11. Now placed in incubator at 37° C.

August 22. Inoculated two glucose-litmus-agar slopes from contents of each container. Placed in incubator at 37° C.

September 3. No growth in any of slopes of 22nd. Experiment concluded.

Result.—M. melitensis will not pass any of Berkefeld filters N., V., or W.

4th Filtration Experiment with M. melitensis.

To see if *M. melitensis* will grow through Berkefeld filters N., V., or W.

One of each porosity taken and treated as in 3rd filtration experiment, and sterilised in autoclave.

August 14. Placed in each bougie with a sterile pipette 5 c.c. of a verified 4 days' broth culture of *M. melitensis*.

August 15. Five more cubic centimetres of same *M. melitensis* broth culture placed in each bougie.

August 16. Filters now working well; V. cylinder being one-third full of filtrate with its bougie immersed in same for ½ inch, W. and N. bougies are only just touching surface of filtrate, so 5 c.c. more of *M. melitensis* broth culture placed in each bougie W. and N.

August 17. N. receiver now half full of filtrate, bougie being

immersed for ¾ inch. More *M. melitensis* broth culture added to W. bougie only.

August 18. W. bougie now well immersed in filtrate. Placed all three in incubator at 37° C.

August 23. Filtrate in N. and W. decreasing in bulk by evaporation through wool plug. Placed more *M. melitensis* broth culture inside these two bougies. Returned to incubator at 37° C.

August 29. Broth filtrates from B., V., and W. have now been incubating at 37° C. for 11 days, bougies being immersed, and remain free from turbidity. Inoculated two agar slopes from each and placed in incubator at 37° C.

September 3. No growth in any of slopes of 29th. Experiment concluded.

Result.—*M. melitensis* will not grow through any of Berkefeld filters N., V., or W.

To Produce a Pure Agglutinating Serum for Testing M. melitensis (*or Growths Suspected to be* M. melitensis) *by Inoculating Rabbits with* M. melitensis.

At first, serum brought by Major Horrocks from Gibraltar, and obtained from a rabbit so inoculated, by him was used for testing all new growths thought to be *M. melitensis*. Later serum obtained from an inoculated monkey, and from the second rabbit in the following three experiments was used :—

1st Rabbit.

June 18. A healthy-looking rabbit was taken, of weight 1310 grammes, and its blood examined for agglutinating action on *M. melitensis*. None was found, and it was injected subcutaneously with ½ c.c. of a 24 hours' growth of *M. melitensis* in broth at 37° C. (verified).

June 25. Agglutination $\frac{1}{10}$ under $\frac{2}{3}$ in obj.

June 28. „ $\frac{1}{10}$ „ and it was injected under skin of back with a 4 days' growth of *M. melitensis* on one agar slope (verified) emulsified in broth.

July 3. Rabbit found dead. *Post-mortem*. There was slight congestion of intestines, spleen, and peritoneal vessels; liver somewhat patchy, heart normal. Stomach full of green food. No *post-mortem* cultures were attempted as animal had apparently been dead 12 to 16 hours.

2nd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope at 37° C., made into an emulsion with 2½ c.c. broth, 1 c.c. of this

injected under skin of back of a fawn and white rabbit weighing 1460 grammes.

July 13. Serum agglutinates in a dilution of $\frac{1}{10}$ *M. melitensis* faintly (microscope $\frac{1}{6}$ obj.); all growth on one agar slope (3 days) of *M. melitensis* (from spleen of man) emulsified in broth and injected subcutaneously.

July 21. Serum in a dilution of $\frac{1}{320}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 24. Serum in a dilution of $\frac{1}{100}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

July 27. Serum in a dilution of $\frac{1}{300}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

Injected growth from two-agar slope of *M. melitensis* (spleen of man), July 27.

July 31. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* ($\frac{2}{3}$ obj.).

August 4. Serum in a dilution of $\frac{1}{1000}$ agglutinates *M. melitensis* visibly to naked eye. Blood had been drawn as required from July 22 onwards.

August 8. Agglutinates *M. melitensis* $\frac{1}{1000}$ visible to naked eye; rabbit now bled to death under ether from carotid by cannula into sterile test-tubes. After separation of serum latter diluted to $\frac{1}{30}$ with sterile salt solution containing $\frac{1}{2}$ per cent. carbolic acid put up in sterile sealed glass capsules and preserved.

Post-mortem.—All organs appear healthy, spleen enlarged. Inoculated to agar slopes each from spleen, liver, kidney, heart's blood and urine.

August 11. Growth on tubes inoculated from *spleen* and *kidneys* verified as *M. melitensis*. No growth on slopes from liver, heart's blood, and urine.

August 13. Still no growth on slopes from liver, heart's blood, and urine. Experiment concluded.

3rd Rabbit.

July 4. Verified 2 days' culture of *M. melitensis* on one agar slope made into emulsion with $2\frac{1}{2}$ c.c. broth, and 1 c.c. of this injected under skin of black and white rabbit, 11 A.M., July 4.

July 9. Serum does not agglutinate *M. melitensis*.

July 13. Serum in a dilution of $\frac{1}{10}$ agglutinates *M. melitensis* ($\frac{1}{6}$ obj. microscope). One agar tube *M. melitensis* from spleen of man emulsified and injected.

July 15. Rabbit died at 4 P.M. A *post-mortem* was made and liver found enlarged and studded with cheesy tubercles the size of peas. Other organs apparently healthy. Two agar slopes inoculated from each. Heart's blood, liver, kidney, and spleen; 2 c.c. of urine taken

from bladder with sterile pipette and put in 19 c.c. broth. All incubated at 37° C.

July 18. No growth on any of slopes; incubated agar slopes from urine broth.

July 19. Growth on slope from urine broth; found to be a short thick bacillus.

July 21. Heart's blood, kidney, liver, and spleen slopes have now been incubated 6 days. No growth on any of them. Experiment concluded.

LONDON :
HARRISON AND SONS, PRINTERS IN ORDINARY TO HIS MAJESTY,
ST. MARTIN'S LANE.